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Physicochemical characterization, microbial decontamination and shelf life analysis of walnut (*Juglans regia* L) oil extracted from gamma radiation treated seeds

Munawar Iqbal [a,*], Ijaz Ahmad Bhatti [b], Muhammad Shahid [c], Jan Nisar [d]

[a] Department of Chemistry, Qurtuba University of Science and Information Technology, Peshawar 25100, KPK, Pakistan
[b] Department of Chemistry, University of Agriculture, Faisalabad 38040, Pakistan
[c] Department of Biochemistry, University of Agriculture, Faisalabad 38040, Pakistan
[d] National Centre of Excellence in Physical Chemistry, University of Peshawar, Peshawar 25120, Pakistan

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ABSTRACT

The physiochemical characterization and microbial decontamination of gamma irradiated (2–10 kGy) walnut seed oil were evaluated. The proximate composition, density and refractive index remained unaffected, whereas unsponifiable matter, iodine, sponification and free fatty acids values were almost unaffected up to an absorbed dose of 6 kGy and slightly changed at higher doses and similar trend was also observed for conjugated dienes, conjugated trienes, peroxide and para-anisidine values. The tocopherol contents and fatty acid also affected at higher absorbed doses. The microbial load was not detected in oil irradiated to an absorbed dose of 6 kGy. It was noted that variations in physiochemical characteristics were insignificant up to 6 kGy. The stored sample did not show significant changes in properties up to 3 months. From the results, it can be concluded that gamma irradiation may affect the physiochemical characteristics of walnut oil at higher doses. Therefore, an appropriate dose of gamma radiation is suggested to avoid any negative impact on nutritional value.

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1. Introduction

Extension in shelf life and food safety is the main purpose of irradiation of foods. Gamma irradiation has become an effective tool for processing and preserving food products and an effective way of controlling food-borne pathogens (Bhatti et al., 2010; Kibar, 2015; Martin et al., 2015; Raisi et al., 2015). Food irradiation is a process in which products are exposed to radiant energy such as gamma rays, electron beams, and X-rays at absorbed doses recommended by the Food and Drug Administration (FDA) for human consumption (Yâlmaz and Geçgel, 2007). Other than environmental application (Abbas et al., 2015; Adesola et al., 2016; Bilal et al., 2014; Babarinde and Onyiaocha, 2016; Iqbal and Bhatti, 2014, 2015; Iqbal, 2016; Iqbal and Khera, 2015; Iqbal and Nisar, 2015; Iqbal et al., 2015; Iqbal, 2016; Iqbal et al., 2015; Jamal et al., 2015; Majolagbe et al., 2016; Pandey et al., 2016a,2016b; Peter and Chinedu, 2016; Qureshi et al., 2015; Sayed, 2015; Ukpaka et al., 2015; Ukpaka, 2016a,2016b), the high energy radiation, especially γ-irradiation has been preferred for infestation control and microbiological safety for food commodities without harming the nutritional value of food commodities (Mexis and Kontominas, 2009; Yaqoob et al., 2010). High doses of radiation sometimes can develop undesirable changes in the flavour, appearance and texture of product, deteriorate the product properties and make them unacceptable for human consumption. For irradiation of food stuffs, different doses of gamma radiation are recommended for commercial food processing (Bhatti et al., 2013). However, nuts contain high levels of unsaturated fatty acids that are more vulnerable to lipid peroxidation upon irradiation. Therefore, it is highly desirable to document the effects of gamma-irradiation on physiochemical characteristics as well as microbial safety of products. Walnut is a highly appreciated nut because of its unique organoleptic characteristics, high levels of polyunsaturated fatty acids (PUFA) and hypocholesterolemic and antihypertensive effects. Walnut kernels have a lipid content of 65% of which 73% consists of PUFA. The high levels of polyunsaturated fatty acids make walnuts highly prone to oxidation. Besides the nature of PUFA, many other factors accelerate the oxidation and deterioration that change the contents including oxygen concentration, relative humidity, light, temperature and microbial contamination (Gecgel et al., 2011). Among these entire factors affecting the nutritional values of food items, the microbial contamination has been recognized as the more effective (Bhatti et al., 2010; Yaqoob

* Corresponding author.
E-mail address: bosalvee@yahoo.com (M. Iqbal).

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et al., 2010). The effect of gamma irradiation on the characteristics of oil extracted from seeds of walnut, grown under local climate has not been reported previously. Therefore, the aim of the present study was to evaluate the effects of gamma radiation treatment on proximate composition, oxidative stability, tocopherol content, fatty acid and microbiological activity of walnut two varieties (hard and soft shell) available in the market, at absorbed doses of 2, 4, 6, 8 and 10 kGy.

2. Materials and methods

All the Chemicals and reagents used were of analytical grade and obtained from Sigma-Aldrich (Buches, Switzerland). Tocopherol standards and fatty acid methyl ester (FAMEs) were purchased from Sigma Chemicals Co (St. Louis, MO, USA).

2.1. Sample collection

Two walnut (*Juglans regia* L.) varieties (soft and hard shell) were collected from the local market, Faisalabad, Pakistan and authenticated from Department of Botany, University of Agriculture, Faisalabad. Walnut seeds were packed in small polyethylene bags, in the form of single layer for irradiation purpose. Each bag was considered as a replicate and three replicates were used for each treatment.

2.2. Gamma radiation treatment

The walnut seeds samples were irradiated at Nuclear Institute of Agriculture and Biology (NIAB) by Cs-137 gamma-radiation source. The samples were irradiated at the absorbed doses of 2, 4, 6, 8 and 10 kGy. Un-irradiated sample was used as control under similar conditions except irradiation. The dose rate at the time of sample irradiation was 1.25 kGy h^{-1} and radiation source was calibrated by Fricke dosimeter system by a relation as shown in Eq. (1).

$$D = \frac{N_A * \Delta A * 100}{\in * \rho * (Fe(III))} \quad (1)$$

Where, D is dose, ΔA is the difference in absorbance of irradiated and un-irradiated sample at 304 nm, N_A is the Avogadro number (6.023×1023 molecule/mole), ϵ is the molar extinction co-efficient of the ferric ions (2205 M^{-1} cm^{-1} at 304 nm at room temperature), ρ is the density of dosimetric solution (1.024 g/cm^3 for 0.4 M H_2SO_4) and G (Fe^{+3}) is the radiation yield (number of ions formed per 100 ev of absorbed energy (15.6 for Fricke solution)) (Sehested, 1970).

2.3. Oil extraction

The oil extraction was done immediately after irradiation. Walnut seeds, both varieties (hard and soft), were grinded using electric grinder and the oil was extracted by Soxhlet apparatus using *n*-hexane as a solvent. Extraction was performed for 8 h and then the solvent was evaporated under vacuum using rotary evaporator (N-N Series) coupled with an aspirator and a digital water bath SB-651 (Eyela, Rikakikai Co. Ltd., Tokyo, Japan) at 45 °C and the extracted oils were stored at 4 °C for further analyses (Wijeratne et al., 2006).

2.4. Seed residues proximate analysis

After oil extraction, both irradiated and un-irradiated walnut seed residues were subjected to proximate analysis following standard methods. Protein contents were determined using the Kjeldahl apparatus (AOAC, 1990). For ash contents determination, 2 g residue was carbonized and ashed in a muffle furnace at 600 °C (ISO, 1977). For the measurement of fiber content, 2 g of defatted sample was boiled with 250 mL of 0.25 M H_2SO_4, separated by filtration and washed to remove insoluble contents. The collected residue was boiled again with 250 mL of 0.313 M NaOH, separated, washed, dried, weighed and ashed at 600 °C using a muffle furnace (Eyela, TMF-2100, Tokyo, Japan) and the loss in mass was determined gravimetrically (AOAC, 1990).

2.5. Physico-chemical characteristics of oils

The IUPAC methods (IUPAC et al., 1987) were adopted precisely for the estimation of iodine, peroxide, density, refractive index, acid, saponification values and unsaponifiable matter of the oils extracted from un-irradiated and irradiated walnut seeds.

2.5.1. Oxidative status of oils

For the measurement of para-anisidine value, IUPAC method (IUPAC et al., 1987) was adopted. The oil samples were dissolved in iso-octane and reacted with 5 mL of a P-anisidine solution (0.25% w/v in acetic acid) for 10 min and the absorbance was measured at 350 nm (CE Cecil, 7200, UK). For the measurement of conjugated diene and triene, oil samples were diluted by iso-octane and the absorbance was measured at λ_{max} 232 nm (conjugated dienes) and λ_{max} 268 nm (conjugated trienes).

2.5.2. Fatty acid profile of oils

According to IUPAC et al. (1987) method, 0.2 g oil was refluxed with potassium methoxide at 50 °C and after transmethylation, the mixture was cooled.The contents were transferred to funnel, shaken well after addition of *n*-hexane. Then it was centrifuged and upper layer containing fatty acid methyl esters was recovered, washed with distilled water and dehydrated with anhydrous sodium sulphate. Analyses were carried out by a Shimadzu gas chromatograph (Shimadzu 17-A). The column used was SP-2330 (Supelco) methyl-lignocerate-coated (polar capillary column (30 m × 0.32 mm) with a film thickness of 0.20 μm). For quantitative determination of the eluted compounds a flame ionization detector was used. Chromatographic conditions were optimized for getting reproducible results. Nitrogen with a flow rate of 3 mL/min was used as carrier gas. Initial oven temperature was kept at 180 °C and increased to final temperature (220 °C) with a ramp rate of 5 °C min^{-1}. Injector and detector temperatures were maintained at 230 °C and 250 °C respectively. The FAMEs were identified by comparing their relative and absolute retention times with pure standards. Chromatography Station for Windows (CSW32) data handling software (Data APEX) was used for quantitative measurements.

Tocopherol content of walnut oil was measured following the method described by Bhatti et al. (2010). High performance liquid chromatography (HPLC) analyses were carried out using HPLC (10A series, Shimadzu, Japan) under following conditions; coupled with a liquid pump LS 10AS, a system controller SCL-10A, a Supelco C18 column (250 × 4.6 mm^2; 5 μm), a fluorescence detector RF-530 and an injector loop of 20-μL (Rheodyne, USA). The column was operated at 30 °C. A mobile phase was mixture of acetonitrile and methanol (1:1 v/v) at a flow rate of 1.3 mL min^{-1}. Calculation of tocopherol was made comparing the area (normalization) with a reference standard.

Oils extracted from un-irradiated and irradiated sample, to the absorbed doses of 2, 4, 6, 8 and 10 kGy were analyzed for the total bacterial and fungal count using method reported by Arici et al. (2007). The oil samples (0.1 mL) were mixed with 10 mL of 2% autoclaved peptone solution. The samples were diluted and plated on media. Nutritional agar (Oxoid, UK) was used for bacterial and

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