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Effect of gamma irradiation on the microbial load, nutrient composition and free radical scavenging activity of *Nelumbo nucifera* rhizome

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ABSTRACT

The assurance of microbial quality is necessary to make plant materials suitable for human consumption and commercialization. The aim of the present study was to evaluate the possibility to apply the gamma radiation treatment on the rhizome samples of *Nelumbo nucifera* for microbial decontamination. The radiation processing was carried out at dose levels of 1, 2, 4 and 6 kGy. The irradiated and control samples were analyzed for microbial load, organoleptic acceptance, extraction yield, proximate composition, phenolic contents and DPPH scavenging activity. The results indicated that gamma radiation treatment significantly reduced microbial load and increased the storability of the irradiated samples. The treated samples were also acceptable sensorically. The extraction yield and phenolic contents increased with the increase of radiation dose. Gamma radiation also enhanced the DPPH scavenging activity.

1. Introduction

Lotus (*Nelumbo nucifera*) is a perennial aquatic crop and belongs to family Nelumbonaceae. It is commonly known as water lily. Apart from the decorative value, the plant is extensively used throughout Asia for medicinal and nutritional purposes (Sridhar and Bhat, 2007). It is widely used in folk remedies as diuretic, astringent, emollient, cooling agent, antiemetic and in the treatment of tissue inflammation, cancer, skin disease, insomnia, leprosy and poisoning nervous disorders (Cour et al., 1995; Sridhar and Bhat, 2007; Yang et al., 2007). Experimental studies on various parts of the plant revealed excellent antioxidant activity (Hu and Skibsted, 2002; Jung et al., 2003; Sohn et al., 2003; Wu et al., 2003). All parts of *N. nucifera* like flowers, flower stalks, young leaves, rhizomes and seeds are edible. In Pakistan, the plant is widely used for its nutritious rhizomes (Khan et al., 1997). The rhizome is the enlarged underwater stem and is usually creamy-white in color. It is cooked as a vegetable and used in salads, soups and desserts. It is found to be rich in dietary fiber, vitamins, amino acids, thiamin, riboflavin, potassium, phosphorus, copper and manganese (Chiang and Luo, 2007; Geng et al., 2007). In a traditional medicine, the lotus rhizomes are used together with other herbs to treat high fever, sunstroke, leucoderma, small pox, diarrhoea, dysentery, cough, dizziness and to alleviate tissue inflammation, cancer and liver cirrhosis (Mukherjee et al., 1997; Sridhar and Bhat, 2007; Yang et al., 2007). These are the major marketable part of the plant. Lotus rhizome is available round the year, but its natural harvest cycle is from mid-summer to late-winter. Currently post-harvest measures of lotus have been given very little consideration by growers and industry in the country.

Plant materials are highly susceptible to microbial contamination (Kneifel et al., 2002) due to the medium (water and soil) in which they grow. The current practices of harvesting, handling, storage and processing may cause additional contamination and microbial growth. Besides, representing a direct health hazard to the consumer, these microbiologically contaminated materials can cause the spoilage of food to which they are added. The microbial quality is extremely important to be achieved according to international requirements (UNIDO, 1984; WHO, 1994) to make plant materials suitable for human use and commercialization. Conventional methods of microbial decontamination were fumigation with gaseous ethylene oxide or methyl bromide, which are carcinogenic and hence are now prohibited or being increasingly restricted in most advanced countries for health, environmental or occupational safety reasons. Gamma irradiation is well known as a decontamination method for many foodstuffs and plant materials (Farkas, 1998; WHO, 1994), being an environment friendly and effective technology to resolve technical problems in trade and commercialization. It can also control a variety of microorganisms and thus improve the quality of plant materials.

To the best of our knowledge, information on the gamma radiation treatment on lotus rhizome is lacking. The purpose of

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the present work was to evaluate the possibility to apply the gamma radiation treatment on *N. nucifera* rhizome for microbiological decontamination and quality improvement. In the current study, we evaluated the microbial load, proximate composition, sensory characteristics, extraction yield, scavenging activity and total phenolic content of radiated and non-irradiated control samples of lotus rhizome.

2. Materials and methods

2.1. Materials

Fresh lotus rhizomes were purchased from the local market in Peshawar, Pakistan. The rhizomes were cleaned, chopped in to pieces and packed in polyethylene pouches (0.021 mm) and sealed with an electric sealer. The pouches were pressed before sealing to remove maximum air. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, gallic acid and Folin–Ciocalteau reagent were purchased from Sigma Chemical Co., USA. All the solvents and other chemicals used were of analytical grade from Sigma Chemicals Co. (USA) and Merck Darmstadt (Germany).

2.2. Gamma irradiation

The packed rhizome samples of *N. nucifera* were irradiated using gamma rays from a cobalt-60 radiation source (ISSLEDOVATEL, USSR) installed at NIFA Peshawar. The dose rate at the irradiation position was 0.90 kGy/h at the time of treatment, as determined with a Fricke dosimeter. The dose levels applied were 1, 2, 4 and 6 kGy. The irradiations were carried out at room temperature and atmospheric pressure. The irradiated and control rhizomes were stored at $4\pm1\,^{\circ}C$ in a refrigerator for subsequent analysis.

2.3. Determination of microbial load

The microbial load of lotus samples was evaluated on day 0, 3, 6, 9 and 12. The total aerobic bacteria were determined by the surface plate agar method with medium containing Difco-nutrient agar 23 g, glucose 5 g, yeast extract 5 g, and K_2HPO_4 2 g/l (pH 7.0). For analysis, two gram of each irradiated and control samples of rhizome was added to 20 ml of 0.01% Tween-20 sterile water and mixed thoroughly. Each suspension was diluted 10^1 to 10^5 times with the same sterile water, and then 0.2 ml aliquots were spread on the surface of agar plate. Total aerobic bacteria were counted after 24 h incubation at 30 °C. Fungal count of the plant was determined also by plate count method using potato dextrose agar. Plates were incubated at a temperature of 28 °C for 3–5 days and counts were recorded in colony-forming units per gram (cfu/g). The presented data were the average counts in three Petri dishes for each test.

2.4. Proximate and chemical analysis

The irradiated and control samples of the plant were analyzed in triplicate for moisture, crude protein, crude fiber and total mineral matter (ash) according to AOAC (1990), immediately following radiation processing. The moisture and total solids were determined in a drying oven at 105 °C until constant weight. Determination of crude fat was carried out using petroleum ether (bp. 40–60 °C) in a Soxtec system HT (Tecator). Estimation of crude protein (% N × 6.25) was performed by the micro-Kjeldahl method. Ash contents were determined by heating the samples at 550 °C and crude fiber by digestion with acid and alkali using Fibertec system (Tecator). Percentage carbohydrate was given by: 100—(percentage of ash+percentage of moisture+percentage of fat+percentage of protein+percentage of fiber). Nutritive value was finally determined by: nutritive value = 4 × percentage of protein+9 × percentage of fat+4 × percentage of carbohydrate. The analysis for ascorbic acid was conducted by titration method using 2,6-dichlorophenol-indophenol.

2.5. Sensory analysis

Sensory analysis was carried out on day 0, 3, 6, 9 and 12. A panel of 10 trained judges (8 males and 2 females) evaluated the samples for appearance and flavor using a 10 points scale, where 0 indicates dislike extremely and 10 like extremely (Larmond, 1977). The ages of the panelist ranged between 26 and 50 years and they were briefed about the experiments before testing the samples. The samples were coded using standard random numbers. The evaluation was conducted at room temperature under normal laboratory light conditions and the panelists were free to judge any sample twice/thrice.

2.6. Extraction

200 gram of the samples were dried in oven at 45 °C, ground and separately extracted in methanol and acetone using a Soxhlet extractor. All the extracts were filtered through Whatman no. 1 filter paper and concentrated under vacuum at 45 °C. The dried extracts were then weighed. Extraction yield for each solvent was calculated by subtracting the dried weight of plant material residues after extraction from the weight of the original plant material. The extracts thus obtained were stored at 4 °C until further analysis for the estimation of total phenolic content and for the free radical scavenging assay.

2.7. Determination of total phenolic content

The total phenolic content of irradiated and control samples of the plant were determined with Folin–Ciocalteau reagent. Appropriate dilutions of the extracts were made. In a test tube, 200 μl of the extract was added to 4 ml of 2% aqueous sodium carbonate solution and mixed thoroughly. Then 200 μl of 50% Folin–Ciocalteau reagent was added to the mixture. The mixture was allowed to stand for 1 h with intermittent shaking and absorbance of the green–blue complex was measured at 750 nm in a spectrophotometer against blank. All spectrophotometric work was performed using the Ultraspec 3000 UV/visible spectrophotometer (Pharmacia). The total phenolic contents were calculated on the basis of the calibration curve of gallic acid. The results were expressed as milligram of gallic acid equivalents per gram (mg/g) of the dry extract.

2.8. Determination of DPPH radical scavenging activity

The antioxidant activity of the irradiated and non-irradiated control samples was determined using DPPH. It is a free radical with a purple color and has a maximum absorption at 517 nm. The free radial scavenging assay is based on the discoloration of the compound when reduced by a free radical scavenger. DPPH scavenging activity was determined at 0.25, 0.50, 1, 2 and 4 mg/ml of the rhizome extract. About 100 μl of the sample at various concentrations was added to 2 ml of DPPH in methanol solution (60 μM) in a test tube and shaken vigorously. After incubation at 37 °C for 35 min in the dark, the absorbance of each solution was determined at 517 nm. The corresponding blank (control) reading was also taken. The activity was expressed as percentage