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Effect of gamma irradiation on the shelf-life and physico-chemical quality of ready-to-cook (RTC) drumstick (*Moringa oleifera*) pods

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ABSTRACT

Effect of radiation treatment (0.5, 1, 1.5, 2 and 2.5 kGy) and storage (10 °C; 15 d) on the quality of RTC drumstick was investigated using full factorial experimental design. The data obtained for total plate count, yeast & mold count, texture, color & sensory quality were fitted into cubic polynomial equations and radiation dose required for maximum storage duration with acceptable microbial load ($< 10^5$ CFUg^{-1}) and sensory quality (overall acceptability > 5) was determined. An improved shelf-life of the product (12 d) with desired sensory and microbial quality was obtained at a dose of 1 kGy. Better retention of phenolics and isothiocyanates in the irradiated product compared to control (non-irradiated) during entire storage duration (12 d) was demonstrated. Presence of isopropyl, 2-butyl and isobutyl isothiocyanate in drumstick pods is demonstrated here for the first time.

1. Introduction

In the past few years there has been a considerable increase in the worldwide demand of convenience foods such as minimally processed fresh-cut vegetables and fruits. Changing lifestyles and preference for fresh, healthy and natural food is a strong marketing drive for these products. These products include Ready-To-Cook (RTC) vegetables or Ready-To-Eat (RTE) fresh fruits that provide convenience by facilitating minimal time for preparation before consumption without substantially altering their fresh like properties and health benefits (Ahvenainen, 1996). Such products possess fresh-like appearance and flavor without the use of preservatives, in addition to convenience. However, the physiological changes and microbial degradation during processing and storage may be accompanied by decay, flavor, color and increased vitamin loss, shrinkage, softening, and thus a shorter storage life. We have earlier demonstrated the effective use of gamma irradiation for improving the shelf life of fresh-cut vegetables such as pumpkin, ash gourd and minimally processed French beans (Gupta et al., 2012; Tripathi, Chatterjee, Vaishnav, Variyar, & Sharma, 2013).

Shelf life of a fresh produce is determined by its microbial status as well as by its sensory attributes for example color, texture and flavor (aroma and taste). Gamma irradiation is known to generate minimal modification in these quality attributes of fresh-cut produce. Studies conducted on irradiated RTC vegetables have demonstrated insignificant changes in the appearance, texture, color, taste and overall acceptability after storage as compared to the control, when stored at low temperatures (4–10 °C). The better quality retention in general, has been attributed to the decreased physiological and microbial decay in the radiation processed samples.

Moringa oleifera belongs to Moringaceae family, and is an important food commodity which has been used to treat malnutrition in nursing mothers and infants. Almost every part of the plant - leaves, flowers and pods are being consumed as extremely nutritive vegetable (Radovich, 2010). Production of drumstick pods in India is a huge trade with approx. 1.2 million MT (metric tons) produced annually (Radovich, 2010). It has been estimated that about one-fourth of all *Moringa oleifera* produce harvested is spoiled before consumption (Prabhu, Rajan, & Santhalia, 2011). Therefore, there is an increasing interest to preserve this vegetable due to its medicinal and therapeutic properties. Studies have been attempted to preserve the shelf-life of leaves and pods of this vegetable using various preservation techniques. Treatment with germicide, salt and turmeric in various combinations and subsequent dehydration by sun drying, oven drying or wind drying was found to effectively improve the shelf life without alteration in the nutritional value (Prabhu et al., 2011). However, no reports exist on the preservation or shelf-life extension of fresh-cut drumstick pods by gamma irradiation and its impact on the shelf-life and quality attributes of the pods.

The mathematical approach for obtaining process parameters for a fresh-cut produce has been shown to be very effective in deciding suitable processing conditions required for desired quality characteristics of the product, and at the same time, reducing severity of the

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treatments. Such equations derived for various fresh-cut produce such as RTC pumpkin cubes (Tripathi et al., 2013) and minimally processed French beans (Gupta et al., 2012) were demonstrated to be quite efficient in deciding the process parameters for desired shelf life and quality parameters. The objective of the current study was to improve the shelf life of ready-to-cook drumstick pods using radiation treatment in combination of cold storage at optimized processing parameters based on full factorial experimental design. An attempt was made to correlate the quality parameters of RTC drumstick with the processing conditions in terms of empirical equations. The impact of radiation processing and storage period was also evaluated on the antioxidant potential and bioactive constituents in the processed product.

2. Materials and methods

2.1. Materials

Drumsticks (*Moringa oleifera*) ranging in moisture content between 85.5 and 88.2% were obtained from a local farmer near Mumbai, India. All the standard chemicals were purchased from Sigma–Aldrich Chemical Company, USA and were used as received. HPLC solvents (EMerck, India) were filtered before use. All the solvents (analytical grade) were distilled before use. Standard allyl isothiocyanate and phenol compounds (gallic, protocatechuic, caffeic and *o*-coumaric acid, catechin, epicatechin, rutin and kaempferol) were obtained from Sigma Aldrich (St. Louis, USA).

2.2. Minimal processing and radiation treatment

Drumstick pods were washed with tap water and cut transversely into pieces of length 4.0 cm approximately. The cut pieces were randomized and aerobically packed (70 g) into polystyrene trays and wrapped around with cling film (Flexo film wraps ltd., Aurangabad, Maharashtra, India). Film used had thickness of 8–10 μm and permeability to carbon dioxide and oxygen as 10.3 and 1.7 $cm^3 m^{-2} s^{-1} Pa^{-1}$ respectively (data as provided by supplier).

The cut and packed drumstick pods were irradiated at different doses (0–2.5 kGy, in an interval of 0.5 kGy) in a cobalt-60 irradiator (GC-5000, BRIT, Mumbai, India) with dose rate of 1.45 Gy/s. The radiation facility was calibrated using Fricke dosimeter and dose uniformity ratio was found to be 1.21. Radiation treated samples were stored at 10 ± 1 °C, RH 65 ± 5%. Non-irradiated samples stored under same conditions were used as control. Samples were prepared in triplicates for each radiation dose and day of analysis. The samples were analyzed for various quality attributes such as microbial and sensory quality, color, texture, DPPH radical scavenging activity, phenolic constituents and isothiocyanate contents on day 0, 5, 8, 12 and 15 after packaging.

2.3. Optimization of quality parameters

A full factorial experimental design was planned using Design Expert 8.0 software (State ease Inc., USA). Experiments were designed for various radiation doses (0, 0.5, 1.0, 1.5, 2.0 & 2.5 kGy) and storage periods (0, 5, 8, 12 & 15) as variables. Samples were analyzed in triplicates from each combination of radiation dose and storage day for total plate count, yeast & mold counts, texture, color, and sensory acceptability.

2.4. Sensory acceptability

Sensory acceptability was analyzed using a 9-point hedonic scale with 1 dislike extremely and 9, like extremely. Cut drumstick pieces were cooked for 3 min in boiling water and examined by a panel of 12 analysts (7 males and 5 females, between 27 and 55 years age) as described earlier (Tripathi et al., 2013). The samples were evaluated for color, texture, taste, aftertaste, aroma and overall acceptability. Radiation treated and stored samples were analyzed with respect to fresh-cut control samples on all days of study. At optimized conditions, sensory evaluation was also performed by quantitative descriptive analysis (QDA) (Supplementary Fig. S1).

2.5. Microbial analysis

Total plate count (TPC) and yeast & mould counts (Y & MC) in control and treated RTC drumstick were determined periodically during 15 d of storage using standard methods. The methodology adopted was same as reported previously (Tripathi et al., 2013). Each analysis was performed in triplicate and microbial counts were represented in terms of $\log_{10} CFU g^{-1}$.

2.6. Determination of color and texture

A colorimeter (CM- 3600d Konica Minolta sensing Inc., Japan) was used to measure the surface values of L* (lightness; 0–100), a* (-green/+red; ± 100), and b* (-blue/+yellow; ± 100), the Commission Internationale de l'Eclairage (CIE) coordinates, as per methodology reported earlier (Gupta et al., 2012). Six drumstick pieces (raw) were randomly evaluated in triplicate from each packaged tray and results were represented as their mean. Firmness was analyzed with a texture analyzing equipment (TA.XTPLUS, Stable Microsystems Ltd., Surrey, England) employing a needle probe (P/2N s/s batch No. 10451) by the procedure of Gupta et al., 2012. The average of peak force required to pierce the samples for replicates from each tray was reported as its firmness.

2.7. DPPH radical scavenging activity and ascorbic acid content

Whole drumstick pod pieces (20 g) were homogenized with 40 mL distilled methanol using an omnimixer (Sorvall, Connecticut, U.S.A). The homogenate was centrifuged (3000 × *g* for 30 min; Eppendorf India, Chennai, India) and the supernatant was made up to 100 ml using methanol. Aliquots thus obtained were used for determining DPPH radical scavenging activity using procedure as reported previously (Tripathi, Gupta, Mishra, Variyar, & Sharma, 2014). The activity was represented in terms of Gallic acid equivalent (mg kg^{-1}) with respect to fresh weight. Ascorbic acid content of drumstick was evaluated as per AOAC standard official micro-fluorometric procedure (AOAC, 1990). Experiments were repeated for three independent samples, each analyzed in triplicate.

2.8. HPLC analysis for phenolics

Phenolic constituents were analyzed using a RP-HPLC system (Jasco, Tokyo, Japan), fitted with a quaternary pump delivery system, a C-18 column (250 mm × 4.6 mm length, 5 μm particle size) and a diode array detector according to the procedure as described earlier (Tripathi et al., 2014). The solvent system used for separation was: solvent A (1.5% *o*-phosphoric acid) and B (acetonitrile:acetic acid:-phosphoric acid:water (20:24:1.5:54.5)). The gradient program used was as follows: 0 min–80% A; 30 min–33% A; 33 min–10% A; and 40 min–0% A. The peaks were identified by comparing retention times and UV-Vis spectra in the 200–500 nm range with those of corresponding authentic standards. Quantification of identified compounds was performed using calibration curves of the corresponding standard compounds at the specific absorption maximum. Co-chromatography with added standards was also performed for further confirmation of identified phenolic compounds.

2.9. Identification of isothiocyanates by GC/MS

50 g of cut drumstick pods from each tray was taken in 85 ml of