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Effect of gamma irradiation under various atmospheres of packaging on the microbial and physicochemical properties of turmeric powder

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ABSTRACT

This study investigated the effect of gamma irradiation (0, 5, 10, and 15 kGy) under various atmospheres of packaging (air, N_2, and vacuum) on the microbial load and physicochemical properties of turmeric powder, including antioxidant activities, total phenolic content (TPC), color parameters, and curcuminoid content. The efficiency of irradiation in reducing microbial contamination in the samples was observed even at the lowest dose. By increasing the irradiation dose, the microbial load was not detectable. Irradiation in the presence of oxygen had synergistic effects on the extraction of curcuminoids and TPC, and increased the antioxidant activity of the methanolic extracts: highest activity was observed at 15 kGy. Generally, gamma irradiation up to the dose of 10 kGy under air atmosphere not only ensured microbial safety and desirability of turmeric powder, but also improved the extraction yield of bioactive compounds and, consequently, antioxidant activities of the samples.

1. Introduction

Turmeric (*Curcuma longa* L.) is a rhizomatous of the Zingiberaceae family, which is famous as a golden spice or life spice. Numerous studies have proven its anti-cancer, anti-diabetic, liver protecting, antioxidant, anti-inflammatory, anti-bacterial, and anti-AIDS benefits (Prasad and Aggarwal, 2011). Because of its beneficial health properties, turmeric spice is known as a functional foodstuff all over the world with various applications in food, pharmaceutical, and health products. Iran is the biggest importer of turmeric spice in the Middle East. According to FAO/IAEA/WHO statistics, around 5000 t of turmeric is imported to Iran annually, showing the high consumption and importance of this spice in different industries in Iran (Asghari et al., 2010). However, since condiments and spices are mainly cultivated in semi-tropical countries such as India, China, Pakistan, and Peru, hot and humid climate as well as poor sanitary conditions of harvesting, storage, and handling have caused them to be considered as the potential sources of microbial contamination (Sádecká, 2007).

Conventional methods are not appropriate for decontamination of spices. Because of thermolabile nature of many essential oil components, heat treatment is not suitable. Also, due to the low penetrating power of ultraviolet radiation, it is not effective in decontamination of great volumes of spices and condiments. Fumigation with methyl bromide, ethylene oxide, and propylene oxide gases is often used for reducing microbial contamination in spices. However, this procedure has recently been prohibited widely across the world—in, for example, Europe, Japan, and the United States—because of toxicity and carcinogenicity concerns as well as safety and environmental issues (Schweiggert et al., 2007).

Ionizing radiation is well-known as a cold-pasteurization method in which no heat is generated. The killing mechanism of ionizing radiation can be divided into direct and indirect effects. Gamma irradiation has now found its place in reducing contamination. It is also known as an appropriate method for reducing food waste after harvesting, ensuring health quality and facilitating the trade of foodstuffs (Sádecká, 2010).

Due to the widespread and growing application of food ionizing radiation, there are contradictory results on its effect on the physicochemical properties of spices and condiments. Therefore, extensive research is essential in this area (Sádecká, 2007).

The optimum dose of gamma radiation for controlling food microorganisms and maintaining qualities of foodstuffs depends on the number of existing microorganisms, resistance of them to radiation, food composition, moisture content, temperature, and presence or absence of oxygen during non-thermal processing (Bendini et al., 1998). The findings of previous studies have demonstrated that undesirable effects of gamma radiation on food products—such as softening, breathing, or lipid oxidation in fresh crops and meat products—are significantly diminished by modified atmosphere packaging (Gunes et al., 2011). However, hydrated foods are more sensitive to ionizing radiation than dried foods like spices (Jung et al., 2015).

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Overall, considering the various nutritional and health benefits of turmeric spice as well as its wide application in food and pharmaceutical industries, and also its high per capita consumption in Iran, there is a lack of information on the effect of gamma irradiation on microbial decontamination and the functional properties of this spice under various atmospheres of packaging. Hence, this research focuses on the effect of gamma irradiation (0, 5, 10, and 15 kGy) under various atmospheres of packaging (air, N_2, and vacuum) on microbial contamination, total phenolic content (TPC), color values, curcuminoids content (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), and antioxidant activities of turmeric powder by three different methods ($DPPH^{\bullet}$, FRAP, and $ABTS^{\bullet+}$).

2. Materials and methods

2.1. Materials

ABTS (2, 2-Azinobis-3-ethylbenzothiazoline-6-sulfonicacid), potassium persulfate, DPPH (2, 2-diphenyl-1-picrylhydrazyl), sodium acetate trihydrate, TPTZ 92, 4, 6-tripyridyl-s-triazine, hydrochloric acid, $FeCl_3{\cdot}6H_2O$, glacial acetic acid, Folin-Ciocalteu reagent, sodium carbonate, HPLC-grade methanol and acetonitrile, culture media such as PCA (plate count agar), VRBL (Crystal violet neutral red bile lactose agar), and YGC (yeast extract glucose chloramphenicol) were purchased from Merck Chemical Co. (Darmstadt, Germany). Water was prepared by double glass distillation and purified with a Milli-Q system (Millipore, Bedford, MA). Standards of curcumin (C), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) were purchased from Sigma-Aldrich (St. Louis, USA).

2.2. Sample preparation and treatments

Rhizomes of turmeric were purchased from a local market in Tehran, Iran. Then their typology (*Curcuma longa* L.) was approved by evaluating their morphological and anatomical properties in the Institute of Medicinal Plants of Shahid Beheshti University (Tehran, Iran). The rhizomes were powdered by hammer mill and fitted with a 0.25 mm sieve (mesh 60). Then turmeric powder (50 g) was packaged by H–ENKELMAN200A (Germany) in modified atmospheres (vacuum and N_2) and air respectively using high-barrier multilayered film (polyethylene terephthalate/ethylene vinyl alcohol-polyamide copolymer/low density polyethylene) (Pet/EVOH–PA/LDPE) with 110 μm thickness, which was purchased from Plastic Machine Alvan Co. (Tehran, Iran).

The packages were put in three same sized cardboards randomly for gamma irradiation at 5, 10, and 15 kGy doses. Then three dosimeters (HARWELL PERSPEX-UK) were put on the surface, center, and bottom of each box in order to determine the average of irradiated dose. The irradiation treatment was carried out to achieve doses of 5, 10, and 15 kGy at room temperature with a ^{60}Co source (activity of 312737 Ci) in the Commercial Irradiation Facility of the Atomic Energy Organization of Tehran, Iran. The irradiation time was 207.6 min for the dose of 5 kGy, 445 min for the dose of 10 kGy, and 553.4 min for the dose of 15 kGy. In addition, the minimum and maximum applied doses for 5, 10, and 15 kGy were 5.4 and 6.8 kGy, 10.8 and 12 kGy, and 13 and 15.8 kGy respectively. Non-irradiated samples were also packaged under different atmospheres, i.e. vacuum, N_2, and air. The samples were then kept in a refrigerator (4 °C) for further analysis.

2.3. Extraction method

Ultrasound assisted extraction (UAE) was done. One gram of each sample was mixed with methanol (Mudge et al., 2016) in the ratio of 1:25 and put in ultrasound bath (TECOGAZ, Italy) for 15 min at 25 °C. It was then centrifuged for 15 min (13,304 × *g*) (ROTINA 35R, Hettich, D-78532 Tuttlingen, Germany). The extracts were dried in vacuum oven after filtration, and, thereafter, their dry weight was determined.

2.4. Microbial analysis

Ten g of each sample was weighed, dissolved in 90 ml of sterile peptone water (0.1%), and homogenized completely with sterile Stomacher. Then serial dilutions were prepared (Jung et al., 2015). Total aerobic bacterial count (Mesophiles) (ISO, 2014), spore forming bacterial count (Banerjee and Sarkar, 2004), total yeast/mold count (ISO, 2008), and coliforms count (ISO, 2006) were determined in triplicate. The results so obtained were assessed, and the appropriate plates containing 30–300 bacterial colonies and 15–150 fungal colonies respectively were selected and counted. Microbial contaminations were also expressed in log cfu/g (Kirkin et al., 2014). Next, D_{10}-value (the dose that reduces a microbial population by 1 log, D) of each microorganism was determined according to Eq. (1) (Song et al., 2014):

$$\mathrm{Log}\frac{N}{N_\circ} = \frac{-1}{D}d \tag{1}$$

where, N and N_0 are the number of survivors at the gamma irradiation dose and the initial population of pathogens respectively; D is D_{10}-value (decimal reduction dose); and *d* is gamma dose. Finally, d (the minimum irradiation dose required to achieve desirable microbial contamination) was obtained by multiplying the D of each microorganism in minimum reduction in microbial contamination (in logarithmic scale) required to achieve desirable microbial load according to the related standard in Iran (Schaarschmidt et al., 2016) as follows:

$$\mathrm{Log}\frac{Ns}{N_\circ} = \frac{-1}{D}\mathrm{d} \tag{2}$$

where, N_s is the number of minimum acceptable microbial contamination limit according to the related standards or seller and buyer agreements which is $\leq 10^2$ for spore forming bacteria (Schaarschmidt et al., 2016) and $\leq 10^3$ for total aerobic bacteria (seller and buyer agreements in Iran) (Mehdizadeh Shahi and Fallahnejad, 2005). N_0 is the initial population of pathogens; and D is D_{10}-value.

2.5. Determination of total phenolic content (TPC)

TPC of the extracts was determined according to the Folin-Ciocalteu method. 20 μl of each extract was mixed with 1.16 ml of distilled water and 100 μl of Folin-Ciocalteu reagent. After 5 min, 300 μl of sodium carbonate 20% was added. The solution was incubated for 30 min at 40 °C, and absorbance of the solution was read at 760 nm by UV–Vis spectrophotometer (Agilent Cary 60, CA, USA) (Slinkard and Singleton, 1977). TPC was expressed as mg gallic acid equivalent per gram dry weight of extract (mg GAE/gdw).

2.6. HPLC analysis of curcuminoids

The curcuminoid content of the extracts was determined by Waters 600 HPLC equipped with a 600E multi-solvent delivery system, with helium degassing, a manual injector with 20 μl loop (Rheodyne 7125), and Waters 2487 dual wavelength absorbance detector. Empower software was used for controlling the analytical system and for data processing. Separations were carried out on a 250 × 4.6 mm, particle size 5 μm, NUCLEODUR® 100–5 C18 column. The elution was done in isocratic mode with a flow rate of 1.0 ml/min at room temperature with running time of 30 min. The mobile phase consisted of acetonitrile and water (50:50). The UV/Vis detector was set to a wavelength of 420 nm. Chromatographic peaks were identified by comparison with retention times of the standards of curcumin (C), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Calibration curves were prepared using each standard at the concentration range of 0.5–400 μg/ml (n = 7) in methanol. Both standards and samples were filtered through a 0.22 μm PTFE filter prior to analysis. The content of curcuminoids (C,