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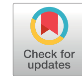
Effect of gamma radiation processing on turmeric: Antioxidant activity and curcumin content



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ABSTRACT

The aim of this study was to evaluate the effectiveness of gamma radiation from ^{60}Co at doses 0, 5, 10, 15 and 20 kGy on turmeric (*Curcuma longa* Linn.). The quantification of phenolic compounds was performed by Folin-Ciocalteu method and assessing the potential of antioxidant activity by the free radical [2,2 difenil-1-pricril-hidrazil (DPPH•)] scavenging and by Rancimat® method. The curcumin quantification was performed by High Performance Liquid Chromatography. Compared to control, there were significant losses ($p < 0,05$) of total phenolic compounds in the samples irradiated with 15 kGy ($p = 0001$) and 20 kGy ($p = 0001$). Regardless the irradiation, there was no decrease in the ability to scavenge free radicals. The Antioxidant Activity Index (Rancimat® method) was significantly lower ($p < 0,05$) in 5 kGy ($p < 0001$) and 15 kGy ($p = 0003$) irradiated extracts and the curcumin quantification was significantly lower ($p < 0,05$) in 15 kGy irradiated extract ($p < 0001$). It is concluded that gamma radiation processing technology on turmeric can be viable. To maintain safety of antioxidant activity it should be applied doses up 10 kGy.

1. Introduction

Fruits and vegetables are, in general, the main source of vitamin C, folate, fibers and bioactive compounds, both of which the human metabolism is dependent. Several species are considered important sources of bioactive compounds and their potential effects against not transmissible chronic diseases have been evaluated in epidemiological studies all over the world (Monsalve et al., 2017; Klinder et al., 2016; Oliveira et al., 2012; Dembitsky et al., 2011). The antioxidant activity is common over the bioactive compounds, especially phenolic compounds, due to: (1) their redox potential; (2) the ability of a particular molecule to compete for active sites and receptors in several cellular structures and (3) the modulating gene expression, which encode proteins involved in intracellular defense mechanisms against oxidative and degenerative processes of cellular structures (DNA, membranes) (Bastos et al., 2009). A great number of species belonging to Zingiberaceae family have antioxidant properties (Chen et al., 2008). Turmeric (*Curcuma longa* Linn.) is the most studied specie of the genus Curcuma. Curcumin (diferuloylmethane) and its derivatives are the most active ingredients responsable for their biological activities, including antioxidant activity, considered the most important of their functional properties (Hussain et al., 2017; Jitoe-Masuda et al., 2013). These bioactive compounds have been recently attracted attention in several areas, such as food, medical and pharmaceutical, due to their multifunctionality (Jitoe-Masuda et al., 2013). Spices and herbal medicines are subject to contamination by microorganisms from soil, air and water. It can be influenced by environmental factors, handling practices and the storage conditions (Araujo and Bauab, 2012). The presence of microbial contamination in plants belonging to Zingiberaceae family, as well as turmeric, is generally high. Even though turmeric powder is obtained from boiled and dried rhizomes, it can contain bacteria in concentration of 10^7 colony forming units per gram (cfu/g) (Rahayu et al., 2016; Yamaoki and Kimura, 2018). Products like turmeric powder need an efficient decontamination method, preferably one that do not leave chemical residues (Haleem et al., 2015). Microbial decontamination with ethylene oxide and methyl bromide are prohibited in several countries due to their damage effects on health and for the environment (Kumar et al., 2010). Furthermore, heat treatments can cause discoloration and reduction of volatile oils contents in spices (Almela et al., 2002; Rico et al., 2010; Sadecka, 2010). Microwave treatment is a possibility, but studies show that it can decrease volatile oil constituents and increase concentration of major terpens relevant to

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flavour of spices (Emam et al., 1995). In this way, many companies have used radiation with high energy to ensure safety of their products without affecting their nutritional quality and promote consumer welfare. Irradiation techniqes compared to other decontamination techniqes are faster, safer, more convenient and eco-friendly (Rahayu et al., 2016). This method can be realized after packaging and provides minimal changes in fresh, perishable and "ready to eat" products. If appropriate doses were applied, they can be preserved for longer without losing its quality (Kirkin et al., 2014; Pereira et al., 2015; Koike et al., 2015; Ehlermann, 2016; Jeong and Kang, 2017). However, foods have some key compounds, which regulate their taste, aroma and nutritional profile that used to be sensitive to irradiation and so much high doses (Kitazuru et al., 2004). In case of turmeric, preservation of curcumin content is important to ensure its flavour as well as its biological activities. Yamoki and Kimura (2018) show that radiosterelization with electro beams is effect and avoid degradation of curcuminoids. Other researches evaluated the effect of gamma irradiation treatment on turmeric samples. This type of treatment appears to be more useful than electro beams to improve food safety (Kim et al., 2010). Doses up to 10 kGy were applied and no changes in curcuminoids content were founded (Chatterjee et al., 1999; Dhanya et al., 2009). Therefore, the aim of this study was to evaluate the effectiveness of gamma irradiation process in doses up to 20 kGy on turmeric, related to quantification of phenolic compounds, antioxidant activity and quantification of curcumin.

2. Materials and methods

2.1. Samples

Non-irradiated, dry and powder samples of turmeric were purchased from SANTOS FLORA COMÉRICO DE ERVAS LTDA, São Paulo, Brazil. The sample was weighed and packaged in polyethylene bags with 1 kg each, sealed and identified with their respective radiation doses. Afterwards, they were storage in ambient temperature.

2.2. Irradiation

The prepacked samples were irradiated in a ^{60}Co multipurpose irradiator, at doses of 0; 5; 10; 15 e 20 kGy/h, in IPEN/CNEN (São Paulo, Brazil) and radiation dose rate was 5,0 kGy/h. Harwell Amber 3042 dosimeters were used to measure the radiation dose.

2.3. Extraction method

The method was performed as described by Chen et al. (2008) with modifications. 0,83 g of turmeric dry sample were weighed and added to 25 ml of acetone/methanol (70:30, v/v). Following this, the mixtures were mixed overnight in a magnetic shaker (Quimis, Q.261.2) and ultrasonicated (Thornton) for 20 min. The samples were centrifuged (centrifugal ALC, 4239R – Italy) at 6000*g* for 15 min. The supernatant was collected and directed to the rotary evaporator and the residue suffered three further extractions. The supernatant resulted from 4 extractions was evaporated and the final volume adjusted to 20 ml of acetone. This process was performed for all proposed radiation doses. The extracts were stocked in amber vials, under nitrogen atmosphere and stored below − 18 °C until the analyze moment.

2.4. Determination of total phenolic content

The total phenolic content of turmeric extracts were determined by the method described by Singleton and Rossi (1965), with modifications. The irradiated and non-irradiated samples were diluted 1:30 for this analyze. A 20 µL aliquot was added to 100 µL of Folin-Ciocalteu and 80 µL of saturated sodium carbonate solution (75 g/L). The reaction mixture was incubated at 37 °C for 30 min in the dark at room temperature for color development. The test was conducted in Spectramax M5 microplate reader (Molecular Devices) and the absorbance was read at a wavelength of 750 nm. Gallic acid was used as standard and distilled water as blank. The total phenolic content was calculated using the equation of a straight line obtained from the gallic acid standard curve (20–100 mg L^{-1}). The results were obtained in mg gallic acid equivalent/g dry sample.

2.5. Antioxidant activity determination by DPPH free radical scavenging activity test (ED 50)

The antioxidant activity of the irradiated and non-irradiated extracts was determined by the spectrophotometric method based on the reduction of 2,2-diphenyl-1-picril-hydrazyl stable free radical, as described by Brand-Williams et al. (1995). In a 20 µL aliquot of extracts in four different concentrations was added 200 µL of DDPH solution (150 µM in MeOH 80% v/v). The reaction mixture was incubated at room temperature for 30 min in the dark. The test was conducted in Spectramax M5 microplate reader and the absorbance decrease was read at a wavelength of 520 nm. The result was obtained in mg/g dry sample required to reduce by 50% the initial DPPH concentration.

2.6. Evaluation of the inhibitory effect on lipid oxidation by the Rancimat® method

The Rancimat method determines the induction period by measuring the increase in the volatile acidic byoproducts released from oxidizing oil or fat at 110 °C. The evaluation of the protective capability on lipid oxidation was made by a Rancimat® 743 apparatus (Methron), connected to the program PC: 743 Rancimat 1.0. This apparatus measured the induction period of 3 g of lard (Sadia) containing 1 mg/ml of turmeric extracts. The temperature was programmed in 110 °C, ΔT = 1,5 °C, airflow of 20 L/h. The tubes were connected to Rancimat apparatus, until the conductivity curve in relation to the induction time (IT) was completed to calculate the Antioxidant Activity Index (AAI). A control was also prepared with lard without antioxidant. BHT at 1,0 mg/ml was used as standard. The result was obtained in Antioxidant Activity Index (AAI), calculated by the equation:

$$AAI = IT\ sample/IT\ control$$

When: IT sample: induction time (h) of lard + extract with sample; IT control: induction time (h) of lard without extract.

Longer induction periods and consequently bigger AAI suggest stronger antioxidant activity.

2.7. Quantification of curcumin

The bioactive compound quantified was curcumin, selected due to the fact that it has a huge importance among turmeric biological activities, mainly with regard to antioxidant activity.

The analyzes were done in liquid chromatography Shimadzu LC-10AD containing DGU-20ª5 degasser, LC-20AT quaternary pump, CTO-20ª column oven, SIL-20AC HT auto-injector, CBM-20ª system controller, SPD M20A diode array detector and software LC Solution version 1,24SPS2. The standard used was Curcumin from *Curcuma longa* (Turmeric) – Sigma-Aldrich. The curcumin determination method was validated. 50 µL of turmeric extract were evaporated under nitrogen atmosphere. The residue was re-dissolved with 1,5 ml of methanol HPLC grade and filtered with 0,45 µm filter (Millipore PVDV). 20 µL of obtained solution were injected into the liquid chromatography in triplicate. The column used was Thermo ODS-2 Hypersil (250 × 4,0 mm, 4 µm) and precolumn Shimadzu GVP-ODS (10 × 4,0 mm, 5 µm), both of them were kept in constant temperature of 30 °C. The isocratic mobile phase was compounded by acetonitrile:deionized water (50:50, v/v), filtered through the membrane (Millipore, PVDF) with 1 ml/min of flow rate. The quantification was performed using external standard

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