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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

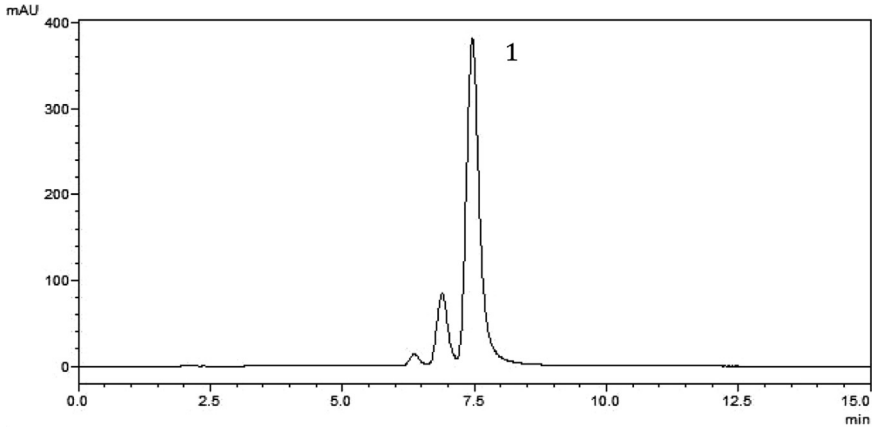

Fig. 1. Chromatogram profile of curcumin standard (1) at 426 nm.

Table 1
Total phenolic content presented in turmeric extracts.

Doses (kGy)	Total phenolic content (mg Gallic Acid equivalents/g dry sample)	
	Mean ± SD	p
0	569,8 ± 20,2	–
5	577,1 ± 24,5	0663
10	536,7 ± 95,1	0521
15	498,4 ± 16,2	0001
20	477,5 ± 18,6	0001

(n) = 4. p < 0,05 shows significant difference compared to control sample (0 kGy).

Table 2
DPPH radical scavenging activity (IC50) of turmeric extracts.

Doses (kGy)	DPPH radical scavenging activity (IC50) (mg/g dry sample)	
	Mean ± SD	p
0	10,8 ± 0,7	–
5	10,3 ± 0,6	0386
10	7,6 ± 1,1	0003
15	10,6 ± 0,3	0635
20	7,5 ± 0,2	> 0001

(n) = 4. p < 0,05 shows significant difference compared to control sample (0 kGy).

Table 3
Antioxidant Activity Index (AAI) in turmeric extracts.

Doses (kGy)	AAI	
	Mean ± SD	p
0	3,43 ± 0,2	–
5	2,65 ± 0,1	< 0001
10	2,72 ± 0,3	0054
15	2,67 ± 0,3	0003
20	3,05 ± 0,5	0182

(n) = 4. p < 0,05 shows significant difference compared to control sample (0 kGy).

and curcumin standard was used in a five-point calibration curve (1,92; 9,60; 24,0; 40,0 e 50,0 μg/ml; r^2 = 0,9998). The identification was made by the retention time and the absorption spectra, relativity of standards. The absorption spectra comparison was made in a wavelength of 426 nm. Fig. 1 showed the curcumin standard chromatography profile.

Table 4
Quantification of curcumin in turmeric extracts.

Doses (kGy)	Curcumin mg/g dry sample	
	Mean ± SD	p
0	858,55 + 11,4	–
5	882,92 + 17,4	0031
10	889,03 + 83,6	0443
15	740,78 + 16,4	< 0001
20	902,78 + 97,6	0344

(n) = 6. p p < 0,05 shows significant difference compared to control sample (0 kGy).

2.8. Statistical analysis

The results are expressed as mean ± SD. The data were analyzed using Student´s *t*-test with significance level of 5% and the analysis was conducted by using SPSS software Version 16.0 for Windows. Significance level of 5% was adopted (p < 0,05).

3. Results and discussion

3.1. Quantification of total phenolic

The quantification of phenolic compounds is shown in Table 1. Samples irradiated by doses of 15 kGy and 20 kGy were the only which showed significant differences in total phenolic content comparing with control sample. These significant losses may be attributed to the formation of radiation-induced degradation products (Sajilata and Singhal, 2006). Breitfellner et al. (2002) showed that gamma radiation in strawberries (1–10 kGy) provided phenolic acids degradation, as well as Schindler et al. (2005), who also observed phenolic compounds degradation in tomatoes irradiated by 2 kGy, 4 kGy and 6 kGy. In the same way, Mahmoud et al. (2016) observed losses in tannins content, another phenolic compound, in millet grains after doses of 2 kGy. Turmeric extracts irradiated by 5 and 10 kGy did not show any significant difference regarding total phenolic content comparing with control and these results are similar with another which has been presented in the literature. Chatterjee et al. (2009) measured total phenolic content in turmeric (*Curcuma longa)* and fenugreek (*Trigonella foneum)* samples and compared with non-irradiated controls. No significant difference was founded. Other studies which evaluated total phenolic content also didn´t show significant losses of these compounds (Harrison and Were, 2007; Lee et al., 2009; Sommer et al., 2009).

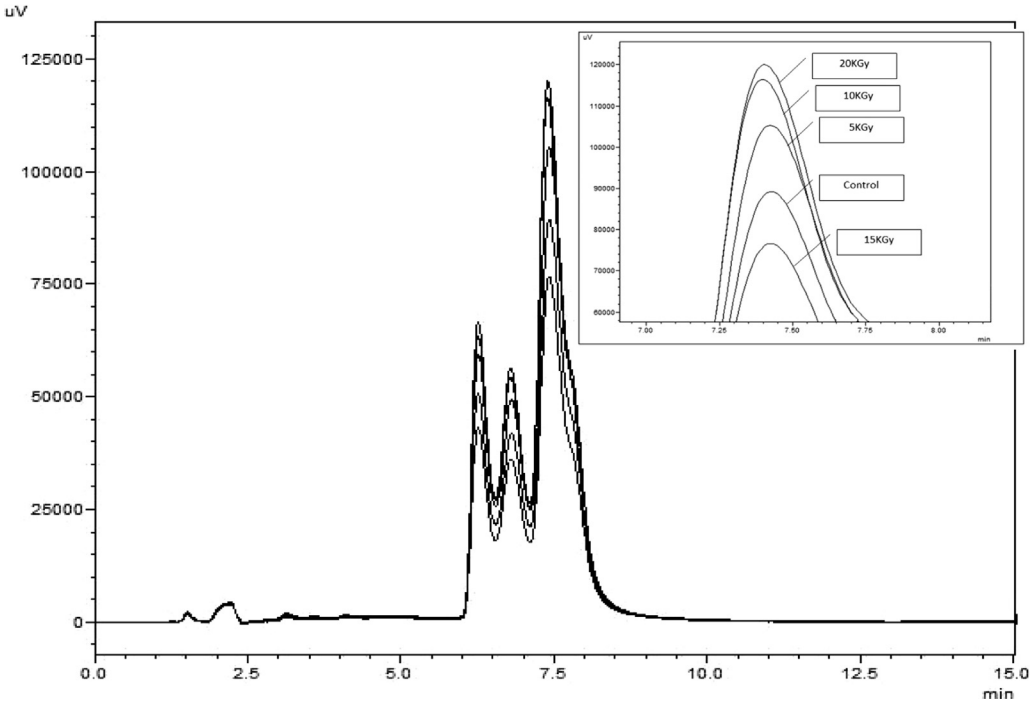

Fig. 2. Comparison between chromatograms profile of turmeric extracts. Control sample; irradiated by 5 kGy; irradiated by 10 kGy; irradiated by 15 kGy; irradiated by 20 kGy.

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

Table 2 shows the 50% radical scavenging activity (IC50) of the extracts studied. Irradiated samples did not show significant losses from control. As observed in our study, Chatterjee et al. (2009) did not find scavenging free radicals significant losses in turmeric samples irradiated by 10 kGy. The inhibition of DPPH activity evaluation showed that extracts irradiated by 10 kGy and 20 kGy had higher antioxidant activity than control and the other samples irradiated by 5 kGy and 15 kGy. Shabana et al. (2017) also verified an increase in antioxidant activity of spiruline (*Arthrospira platensis*) irradiated by 2 kGy and Koike et al. (2015) verified an increase in antioxidant activity of capuchin flowers (*Tropaeolum majus* L.) irradiated by doses up to 1 kGy. In fact, literature usually shows conflicting results regarding the gamma radiation influence on antioxidant activity of foods. According to current studies, radiation treatments have been shown to either increase or decrease the antioxidant activity of a plant, so as not to influence its capacity of scavenging free radicals (Alothman et al., 2009). Phenolics are one of the most responsible about food antioxidant activity when it is measured by scavenging free radicals. However, the quantification of these compounds is not the only way to evaluate this characteristic, owing to the fact that the antioxidant activity also depends on the number and location of hydroxyl radical in aromatic ring, so as their mutual positions (Sommer et al., 2009). Otherwise, phenolic compounds are not free at food matrix, but linked to carbohydrates, proteins and other biomolecules and, in this way, they can be not totally quantified (Fanaro, 2013). It can explain the difference between this test and the total phenolic content test. There was a variation in the antioxidant activity of turmeric extracts in different radiation doses. This effect is also observed in other studies and, under our conditions, it is not possible relate this increase or decrease directly to radiation doses. Literature is scarce in explaining why this phenomenon occurs (Fanaro, 2013). Fanaro (2013) found a drop in antioxidant activity of green tea (*Camellia sinensis*) irradiated by 1,5–2,0 kGy compared with control and an increase at samples irradiated by 2,5 kGy. According to this author, the food matrix and the water activity seems to play an important role in the random interaction of irradiation on food (Fanaro, 2013). In this case, the relative humidity of turmeric extract before the irradiation was 12%.

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

The extract´s AAI and their comparison with control are expressed in Table 3. All tested turmeric extracts showed antioxidant activity and there were significant losses in extracts irradiated by 5 kGy and 15 kGy comparing with control. In this test, the standard BHT presented an AAI of 3,72. The results of this study are similar to what has been published in the literature. Murcia et al. (2004) evaluated the influence of irradiation procedure on antioxidant activity of many spices and the method used was also the Rancimat® test. Irradiated samples (1, 3, 5 and 10 kGy) did not show significant differences ($p < 0,05$) in antioxidant activity comparing to non-irradiated samples. Irradiation insignificant effect on dried plants composition may be explained by its low water composition, which limited the possibility of free radicals formation and its interference on irradiated samples antioxidant activity (Venskutonis et al., 1996). Degradation of phenolic compound and other phytochemicals responsible for antioxidant activity may explain the significant difference obtained at turmeric irradiated by 5 kGy and 15 kGy tests, as discussed in previous items. The variation founded in AAI was also discussed in previous items.

3.4. Curcumin quantification

A significant difference of 5% ($p < 0,05$) in extracts irradiated by 15 kGy was observed, which represent lower concentration of this bioactive compound on this extract (Table 4). This result agree with total phenolic content test and Rancimat® test: turmeric extract irradiated by dose of 15 kGy showed significant differences in these tests comparing to the control, i.e. lower total phenolic content and lower antioxidant activity. This way, it is possible to verify the curcumin content importance in the antioxidant activity of turmeric. As a phenolic compound, it was expected curcumin hydroxylations after high

doses of radiation and it can be attributed to the production of hydroxycyclohexadienyl radicals (OH-adducts) during the irradiation treatment. Chosdu et al. (1995) showed in their studies that gamma irradiation at dose of 10, 30 and 50 kGy induced the producing of free radical in turmeric powder, as well as in pure curcumin. Addition of oxygen to theses OH-adducts followed by elimination of HO_2 radicals leads to hydroxylation of the aromatic ring (Schindler et al., 2005). Despite significant losses presented in extract irradiated by dose of 15 kGy, the chromatography profiles did not show differences among the samples studied, showing the same elution order and retention times. Fig. 2 shows the comparison among the chromatographies studied. Gamma radiation at 10 kGy did not affect curcuminoids quantification in turmeric samples, such as curcumin, demethoxy curcumin and Bis demethoxy curcumin in studies of Chatterjee et al. (1999). Researches realized with capuchin flowers (*Tropaelum major* L.) did not observe differences in quantification of antocianins, also a phenolic compound, after gamma irradiation up to 1 kGy (Koike et al., 2015). However, Pereira et al. (2015) found by means of HPLC an increase of phenolic compounds extratification in *Ginkgo biloba* aqueous extract irradiated by 10 kGy, compared to non-irradiated ones and they considered the breakdown of chemical bonds, caused by irradiation, responsible for the result. This way, we can consider that it happened also in our study with turmeric extract irradiated by 20 kGy, since curcumin content at this extract had an increase compared with extract irradiated by 15 kGy.

4. Conclusion

In the present study, we can conclude that gamma radiation from ^{60}Co as a processing technology can be viable in turmeric (*Curcuma longa* Linn.). To maintain safety of antioxidant activity it should be applied doses up to 10 kGy since higher losses was found in samples irradiated in doses of 15 kGy and 20 kGy.

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