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EFFECT OF THE RADIATION PROCESSING ON THE ANTIOXIDANT ACTIVITY OF ZINGIBERACEAE FAMILY PLANTS

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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 the antioxidant activity of Zingiberaceae plants. For this study were selected: turmeric (*Curcuma longa* L.), ginger (*Zingiber officinale* Roscoe) and zedoaria (*Curcuma zedoaria* (Christm.) Roscoe). The following methodologies applied were: quantification of phenolic compounds 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 in acetone:methanol extracts of selected vegetables. Regardless of the radiation dose applied there were no decrease of total phenolic compounds compared to the control, in any plant studied. The results did not show a decrease in the ability to scavenge free radicals in turmeric case and in the case of zedoaria it were decreased only in doses of 20 kGy. Non-irradiated ginger extract showed higher ability on scavenge. The analysis of the antioxidant potential by Rancimat® method showed no significant difference in the antioxidant activity index (AAI) between doses applied in turmeric and ginger extracts. Already, zedoaria non-irradiated extract showed significantly higher AAI than those presented by irradiated ones. Major losses in the potential of antioxidant activity were found in doses of 20 kGy. It could be concluded that gamma radiation processing of Zingiberaceae plants in doses until 15 kGy may be a feasible alternative to industry, do not change the quantitative profile of phenolic compounds or decrease its expressive antioxidant potential.

1. INTRODUCTION

The Zingiberaceae family is the largest of Scitaminae order and belongs to Monocotiedoneae class. They are characterized for their tuberous or no tuberous rhizome [1].

The Zingiberaceae family plants mainly present essential oils, including terpenes, alcohol, ketones, flavonoids, carotenoids and phytoestrogens. Phenolic constituents like curcuminoids and gingerols isolated, which have had reports of biological activities such as antioxidant, antifungal, insecticidal and anti-inflammatory activity, are particularly important and relevant for their applications [2,3,4].

It is known that many species of the Zingiberaceae family have antioxidant properties [5]. The consumption of food and medicinal plants which have this bioactivity is particularly important, since Reactive Oxigen Species (ROS), also called free radicals, generated by

physiological events, such as cellular respiration, can cause changes in cells when act directly on some cellular components [6].

Antioxidant compounds (such as phenolic compounds) can be identified as beneficial to health because the ability to react with ROS and prevent or minimize the damage they can cause in the body [7,8].

The imbalance between the ROS production and antioxidant defense system can lead to some pathological states [9,10]. Some of pathological processes possibly triggered by the action of ROS are: cancer, inflammatory processes, arthritis, atherosclerosis, cerebral and coronary ischemia, diabetes, Parkinson´s disease, shock, in addition to cellular accelerated ageing [11].

Plant materials are highly susceptible to microbial contaminate 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 [12].

In this way, irradiation with ionizing radiation is one of the most effective means to disinfecting and preserving foods and herbal medicines. Besides disinfecting, irradiation is also used to prolong shelf life as well as delaying ripening and retarding sprouting from the roots of fruit and vegetables [13,14].

Many companies of food and herbal medicine industry use irradiation to ensure safety of these products. Irradiated powder and extracts of Zingiberaceae family plants are sold to food and pharmaceutical industry. It is therefore important to evaluate if irradiation with ionizing radiation modifies their functional properties.

The aim of this study was to evaluate the effectiveness of gamma radiation from ^{60}Co on the antioxidant activity of Zingiberaceae plants. For this study, we selected three plant species with expressive use in Brazil: Turmeric (*Curcuma longa* L.), Ginger (*Zingiber officinale* Roscoe) and Zedoaria (*Curcuma zedoaria* (Christm.) Roscoe).

2. MATERIAL AND METHODS

2.1 Samples

Samples of dry and non-irradiated turmeric and ginger were purchased from SANTOS FLORA COMÉRICO DE ERVAS LTDA (São Paulo, Brazil). The zedoaria, also dry and non-irradiated, was donated by the same company. As the awards given, the turmeric was from Turkey, ginger was from Brazil and zedoaria was from India.

2.2 Irradiation

The samples were packed in plastic (polyethylene) bags, sealed and identified with their respective radiation doses. They 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). Harwell Amber 3042 dosimeters were used to measure the radiation dose.

2.3 Preparation of extracts

The methodology was performed as described by Chen *et al* (2008) [15] with modifications. 0,83g of dry samples of studied plants were weighed and 25mL of solvent were added. The solvent for turmeric was acetone:methanol (70:30 v/v), for ginger and zedoaria was acetone:methanol (50:50 v/v). Then, the mixtures were mixed overnight in a magnetic shaker (Quimis, Q.261.2) and ultrasonicated (Thornton) for 20 minutes. The samples were centrifuged (centrifugal ALC, 4239R – Italy) at 6000g for 15 minutes. The supernatant was collected and directed to the rotary evaporator and the residue suffered three further extractions.

2.4 Determination of soluble solids

The soluble solids were determined gravimetrically. Test tubes were placed in an oven at 105^{o}C overnight and left overnight for complete evaporation of water. On the next day the tubes were removed from the oven, cooled in a desiccator and weighed on an analytical balance. The difference between the value of the initial and final weight is the weight of soluble solids present in 1 mL of extracts. The result was obtained in mg / mL of extract [16].

2.5 Total phenolic determination

The total phenolic content of Zingiberaceae extracts was determined by Folin-Ciocalteau method, as described by Genovese et al. [17] with modifications. After extracts dilution, a 20µL aliquot was added to 100µL of Folin-Ciocalteu and 80µL of saturated sodium carbonate solution (75g/L). The reaction mixture was incubated at 37^{o}C for 30 minutes 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 result was obtained in mg gallic acid equivalent / g dry sample.

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

The capacity of Zingiberaceae to remove 2,2-diphenyl-1-picril-hydrazyl radical was determined by the spectrophotometric method described by Brand-Williams et al. [18].

In a 20µL aliquot of extracts in four different concentrations was added 200µL of DDPH solution (150µM in MeOH 80% v/v). The reaction mixture was incubated at room temperature for 30 minutes in the dark. The test was conducted in Spectramax M5 microplate reader (Molecular Devices) 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.

A calibration curve was prepared from a DPPH solution in 0,06 mM in methanol (MeOH) 80% v/v in 0, 10, 20, 30, 40, 50 e 60 µM/mL.

2.7 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 3g of lard (Sadia) containing 1 mg/mL of turmeric, ginger and zedoaria extracts.

The temperature was programmed in 110°C, ΔT = 1,5°C, airflow of 20L/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 formula:

$$AAI = \frac{IT\ sample}{IT\ control}$$

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.

2.8 Statistical Analysis

The data were analyzed using one-way ANOVA and Tukey test with significance level of 5%.

3. RESULTS AND DISCUSSION

3.1 Total phenolic determination

The phenolic compounds of extracts studied quantification is shown in Table 1.

Table 1: Effects of γ-radiation on phenolic compounds quantification of Zingiberaceae plants

Doses (kGy)	Phenolic compounds quantification (mg gallic acid equivalent / g dry sample)		
	Turmeric	Ginger	Zedoaria
0	570,3 ^{a} ± 20,2	251,6 ^{a} ± 14,4	106,4 ^{a,b} ± 3,3
5	577,1 ^{a} ± 24,5	237,6 ^{a} ± 8,5	101,3 ^{a} ± 4,9
10	536,7 ^{a} ± 95,1	246,3 ^{a} ± 52,1	112,1 ^{a,b} ± 11,4
15	498,4 ^{a} ± 16,2	252 ^{a} ± 21,5	119,7 ^{b} ± 2,5
20	476,72 ^{a} ±18,6	286,5 ^{a} ± 6,8	102 ^{a} ± 8,4

(n) = 4. Different lowercase letters in the same column means a statistical difference ($p<0.05$).

Regardless of irradiation dose applied no significant difference in phenolic compounds quantification was found, in any of studied plants, comparing to control.

Villavicencio et al [19] evaluated the effect of irradiation in Brazilian beans and showed similar results. Furgeri et al. [20] demonstrated that the irradiation at doses up to 10kGy had no effect on phenolic compounds in maté (*Ilex paraguariensis*). Other studies that evaluated the effects of irradiation in vegetables showed the same result [21, 22].

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

Table 2 shows the antioxidant activity determination by DPPH method in the extracts studied.

Table 2: Effects of γ-radiation on DPPH inhibition activity (ED 50) of Zingiberaceae plants

Doses (kGy)	DPPH inhibition activity (ED 50) (mg / g dry sample)		
	Turmeric	**Ginger**	**Zedoaria**
0	$11,28^{b} \pm 0,92$	$15,7^{a} \pm 1,28$	$93,86^{b} \pm 3,05$
5	$10,32^{b} \pm 0,56$	$24,96^{b} \pm 0,77$	$79,19^{a} \pm 3,3$
10	$7,56^{a} \pm 1,34$	$25,17^{b} \pm 3,1$	$86,82^{a,b} \pm 1,6$
15	$10,56^{b} \pm 0,4$	$29,63^{b} \pm 1,19$	$78,04^{a} \pm 3,3$
20	$7,52^{a} \pm 0,18$	$42,67^{c} \pm 3,09$	$135,36^{c} \pm 6,3$

(n) = 4. Different lowercase letters in the same column means a statistical difference ($p<0.05$).

Compared to control, there was no decrease in the ability to sequester free radicals in irradiated turmeric and in the case of zedoaria there was a decreased only at dose of 20kGy. Ginger non-irradiated extract showed higher ability on scavenge, but this lost on antioxidant activity can be considered minimal due to its high antioxidant capacity. Major losses were found at dose of 20kGy.

Furgeri et al. [20] demonstrated that the irradiation at doses up to 10kGy had no effect on phenolic compounds in *tererê*, a typical South America beverage make with maté (*Ilex paraguariensis*). Just as this study, Jo et al [23] also obtained significant differences with green tea irradiated at dose of 20 kGy.

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

The AAI of Zingiberaceae plants studied are expressed on table 3.

Table 3: Effects of γ-radiation on AAI of Zingiberaceae plants

Doses (kGy)	AAI		
	Turmeric	**Ginger**	**Zedoaria**
0	$3,33^{a} \pm 0,2$	$3,875^{a} \pm 0,42$	$2,48^{a} \pm 0,26$
5	$3,05^{a} \pm 0,1$	$4,242^{a} \pm 0,33$	$1,8^{b} \pm 0,13$

10	2,72 [a] ± 0,25	4,233 [a] ± 0,48	1,89 [b] ± 0,13
15	2,67 [a] ± 0,28	3,973 [a] ± 0,31	1,89 [b] ± 0,16
20	3,05 [a] ± 0,58	4,160 [a] ± 0,25	1,81 [b] ± 0,27

(n) = 4. Different lowercase letters in the same column means a statistical difference ($p<0.05$).

No significant difference was found on AAI of irradiated turmeric and ginger comparing to control. Already, control zedoaria presented higher AAI than irradiated ones (this could be attributed to radiation induced disruption of the cell wall structure and consequent higher extractability from the tissues). Major losses were found at dose of 20kGy.

Murcia et al [24] compared the antioxidant activity of seven spices irradiated and non-irradiated by Rancimat® method. Irradiation at doses until 10kGy did not show any significant influence on the antioxidant activity of some spices such as nutmeg and anise.

Ginger showed higher AAI than the other Zingiberaceae studied, eve than BHT (AAI= 3,72). He et al [25] found that ginger had a better antioxidant effect than cinnamon in lard and peanut oil, probably due to the presence of tocopherols, phospholipids and phenolic compounds, which have aromatic rings, or due to the synergistic effect of these compounds.

4. CONCLUSIONS

It could be concluded that gamma radiation processing of Zingiberaceae plants (turmeric, ginger and zedoaria) in doses until 15 kGy may be a feasible alternative to industry, do not change the quantitative profile of phenolic compounds or decrease its expressive antioxidant potential. Thus, it can be used as a preservative technique.

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