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Effects of electron beam irradiation on the bioactive components of goji-berry

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ABSTRACT

The goji-berry (*Lycium barbarum*) is an oval-shaped orange-red fruit with a slightly sweet flavor. This berry has a high antioxidant potential and presents interesting nutritional and therapeutic properties. Irradiation is a safe method that has long used to reduce the microbiological contamination of dried and dehydrated food products. This study aimed to evaluate the irradiation effects on the bioactive compounds of goji berries by irradiating samples with an electron beam at doses of 2.5, 5.0, 7.5, and 10.0 kGy. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and oxygen radical absorbance capacity (ORAC) were assessed by analyzing the hydro-alcoholic extracts. The total phenolic compounds determined by the *Folin-Ciocalteu* assay. Doses up to 10.0 kGy did not significantly affect the antioxidant activity in the DPPH assay, and electron beam irradiation improved the total antioxidant activity of the samples in ORAC, as well as a total flavonoid and phenolics assays.

1. Introduction

Goji-berry *(Lycium barbarum)* fruits are orange-red, with a sweet-and-tangy flavor, and oval shape (3–8 mm in diameter and 6–20 mm in length) (Kulczyński and Gramza-Michałowska, 2016; Amagase and Farnsworth, 2011) member of the Solanaceae family, found mainly in the region of Tibet, China and also in Mongolia (Gross et al., 2006).

The goji-berry has been used as food and traditional Chinese medicinal herb and currently, it has become a popular food in East and West (Donno et al., 2015; Zeng et al., 2019). Studies indicate the beneficial effects of goji-berry for maintaining human health as glucose control in diabetics, anti-tumor activity, anti-oxidative stress, glaucoma, anti-aging and anti-inflammatory (Amagase and Farnsworth, 2001; Tian et al., 2019; Zeng et al., 2019).

Researchs have confirmed the presence of substances with antioxidant activity in *L. barbarum*, mainly vitamin C, carotenoids (zeaxanthin, lutein, and carotene) and high content of polysaccharides (Amagase and Farnsworth, 2011; Sangiovanni et al., 2017). Studies carried out on diabetic animals (rats and mice) treated with goji-berry polysaccharide increased activity of antioxidant enzymes, reduced the rate of cellular oxidative stress, and a significant decreased in the concentration of blood glucose levels (Zhao et al., 2009; Jing et al., 2009).

Protti et al. (2017) studied the antioxidants and total antioxidant capacity of goji-berry using methods (ORAC and DPPH) and resultants obtained from antioxidant capacity assays confirmed the highest nutritional and commercial value of goji-berry.

However, goji-berry can be marketed in the dried form, freshly squeezed for their juice, and concentrated for beverages, requiring methods that guarantee its safety and extend the shelf life. The process of food irradiation is an effective tool in preserving and extending the shelf life of perishable products, insect disinfestation, improving sanitary quality and food safety, can be used to treat a wide variety of foods (Villavicencio et al., 2007; Farkas and Mohácsi-Farkas, 2011; Ehlermann, 2016).

According to Villavicencio et al. (2018), the electron beam

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irradiation technology is an effective process to be applied to food preservation and recommended doses does not affect the quality and integrity of foods in terms of sensory, nutritional and bioactive compounds. E-beam treatment has several benefits compared to cobalt-60 irradiation, for example, by easy handling and low degradation effect to the irradiated product (Watanabe, 2000).

E-beam treatment uses an electrical source instead of radioisotopes to generate ionizing energy (Farkas and Mohácsi-Farkas, 2011). Furthermore, the electron beam machine has the possibility of being disconnected when not in use, this source does not need reloading, is easily available and has a high dose rate, streamlining the process and reducing logistics cost (Villavicencio et al., 2007).

Therefore, radiation treatment of food has been effective for food conservation and safety. The aim of this study was to investigate the effects of ionizing radiation at doses of 2.5, 5.0, 7.5 and 10.0 kGy in the antioxidant activity of goji-berry.

2. Experimental

2.1. Samples

Samples of goji-berry were collected from local supermarkets in the downtown area of São Paulo, Brazil. They were transported to the laboratory in polypropylene bags and stored in a refrigerator with a temperature of approximately 5 °C. Samples were selected and after all were packed in polyethylene plastic bags, sealed and labeled with their respective radiation doses.

2.2. Sample irradiation

The samples were irradiated at Nuclear and Energy Research Institute - IPEN/CNEN, addressed in São Paulo, Brazil), using an electron beam accelerator (IBA Industrial Inc., Edgewood, NY, USA), at room temperature (25 °C) an energy of 1.5 MeV, a dose rate of 5.58 kGy/s, beam current: 1.4 mA and tray speed: 6.72 m/min. The applied doses were ± 2.5 kGy; ± 5.0 kGy; ± 7.50 kGy and ± 10.0 kGy and the dose uniformity ratio was 1.25 kGy. The dosimetry was with ESR/Alanine system (Kuntz et al., 2015). Unirradiated samples served as controls.

2.3. Obtaining extracts

The hydroalcoholic extract was prepared from the methodology adapted as described by Barros et al. (2008) and Kaisoon et al. (2011). The hydroalcoholic extract was made from a solvent mixture of methanol and water (80:20, v/v).

Then, 1 g of fruit sample was weighed into a flask and 30 ml of methanol/water solution was added. After that, the sample was stirred for 1 h, filtered in Whatman funnel (paper No. 40), and stored in a volumetric flask. The residue was resubmitted to the process of stirring, filtration, and storage. The sample in the flask was placed on a rotary evaporator Fisatom brand (model 801) for 4 h until complete evaporation of the methanol. The resultant was stored in packages, frozen and lyophilized (Solab SL404, São Paulo, Brazil).

2.4. Antioxidant activity

2.4.1. DPPH - radical scavenging activity assay

The free radical scavenging efficiency of the isolated compounds was determined by the declaration of the DPPH radicals (2,2-diphenyl-1-picrylhydraz). The DPPH radical–scavenging activity was conducted according to the methodology adapted from Brand-Williams et al. (1995).

The reaction mixture consisted of different concentrations (0.5 ml) of the goji-berry extracts solutions and methanolic solution (1.5 ml) containing DPPH radicals (6×10^{-5} mol/l), which were placed on the tubes and the absorbance was measured by a spectrophotometer (UV-1601, Shimadzu). The mixture had stood in the dark for 30 min, and the absorbance was measured at 517 nm (spectrophotometer mentioned above).

The radical scavenging activity (RSA) was calculated using trolox to obtain the standard curve and the results were expressed as mol trolox equivalent per grams of dry extract. All measurements were carried out in triplicate and the results were averaged. The percent scavenging capacity was calculated using the following equation. AS is the absorbance of the solution containing the sample and ADPPH is the absorption of the DPPH solution. The antioxidant capacity to scavenge DPPH was expressed as Trolox equivalents (TE) *i.e.* μM TE/ml.

$$RSA\% = [(ADPPH - AS) / ADPPH] \times 100$$

2.4.2. Oxygen radical absorbance capacity assay – ORAC

The ORAC method, adapted by Chisté et al. (2011), based on the reaction of AAPH solution [2,2′-azobis (2-amidinopropane)] which is a free radical generator with fluorescence. 15 mg was selected of the extract samples and the samples were diluted in a 1.5 ml phosphate buffer solution; the test was at 96-well microplate (black color). Therefore, to each was added 30 μl of sample, 60 μl of the fluorescein solution at 508.25 nM, and 110 μl of AAPH solution at 76 mM. The phosphate buffer was used in the control (200 μl). It was measured fluorescence microplate reader Synergy HT (Bio-Tek Instruments, Inc, Winooski, VT, USA), where the microplate was incubated at 37 °C with stirring for 2 h, the fluorescence emission was 528 nm and excitation 485 nm. Fluorescence was recorded every minute until it reaches zero.

Assays were performed in triplicate. The evaluation of the ORAC value was made by calculating the value of the area under the samples of the curve (AUC) and expressed in micromol μtrolox equivalent per gram of dry extract.

2.4.3. Total phenolics assay

Total phenolic compounds were determined by the *Folin-Ciocalteu* reagent (Abu Bakar et al., 2009). The extract solutions (0.5 ml) were mixed with *Folin-Ciocalteu* (Sigma-Aldrich, St. Louis, EUA) at 10% reagent (2.5 ml) and sodium carbonate at 4% (2 ml). The tubes were allowed to stand for 2 h in the dark for color development. Absorbance was then measured in a quartz cuvette at a wavelength of 740 NM by a spectrophotometer (UV-1601, Shimadzu).

Assays were performed in triplicate. Gallic acid (Sigma-Aldrich) was used to obtain the standard curve and the reduction *Folin-Ciocalteu* reagent by the samples were expressed as milligrams of GAE (Gallic acid equivalent)/g of dry extract (Tuck and Hayball, 2002; Oliveira et al., 2008). The results were expressed as milligrams GAE per 100 g of sample (mg GAE/100 g).

2.4.4. Total flavonoid assay

The flavonoids content was determined by the $ALCL_3$ assay developed by Zhishen et al. (1999). Initially, 0.2 ml of sample were mixed with 0.4 ml of distilled water. Followed by the addition of sodium nitrite at 5% (0.06 ml), then the mixture was shaken, and left to rest for 5 min. Aluminum chloride solution at 10% (0.6 ml) was added, shaken and left to rest for more 5 min. The next step was the addition of sodium hydroxide at 4% (0.4 ml) and distilled water (0.34 ml). Then the final mixture was left to rest for 15 min and the absorbance was measured at 510 nm by a spectrophotometer.

Assays were performed in triplicate. (+)-Catechin was used to calculate the standard curve. The results were expressed as milligrams of (+)- catechin equivalent (CE) per gram of dry extract.

2.5. Statistical analysis

Data from all methods were analyzed by one-way analysis of

variance (ANOVA) was carried out to evaluate the significance statistical differences between the samples analyzed followed by Scott-Knott's test at 5% ($p \leq 0,05$) of statistical significance. For all statistical analyses, R software, version 3.5.1 was used. The results were expressed as mean ± standard deviation.

3. Results and discussion

3.1. DPPH - radical scavenging activity assay

The EC_{50} values in the DPPH assay ranged from 0.180 to 0.186 mg/ml. Indeed, for a better comparison of the antioxidant activity. Regarding DPPH assay, the EC_{50} is the antioxidant concentration required to obtain a 50% radical inhibition.

In research carried out about radiation influence on the antioxidant activity food, a variation in the number of antioxidant substances present in the samples is observed according to the applied dose. However, this variation is not significant in relation to the non-irradiated samples as reported by Fernandes et al. (2014), Koike et al. (2015) and Pereira et al. (2015).

In the same way, no significant statistical difference ($p > 0.05$) among the doses and non-irradiated samples was observed in this study (Table 1), indicating that radiation by electron beam did not affect the free radical scavenging activity of goji-berry samples. Similarly, studies of *Camellia sinensis* and with phytotherapy, demonstrated that the radiation up to 10.0 kGy has no interference with an antioxidant in this kind of food (Mishra et al., 2006; Kumar et al., 2010; Pereira et al., 2015; Mladenova et al., 2019).

3.2. Oxygen radical absorbance capacity assay – ORAC

ORAC assay was employed to evaluate the total antioxidant capacity. This method can explore a wider aspect of the antioxidant properties of the obtained extracts. ORAC method was employed to represent the significant physiologically radicals and indicated the ability to capture radicals through hydrogen atom transfer.

The results of the ORAC ranged from 135.70 to 190.13 μmol TE/100 g. Every sample irradiated present higher values and presented a significant difference ($p < 0.05$) to non-irradiated. Based on Table 2, we can verify that the antioxidant activity of the irradiated samples up to 2.5 kGy was a more positive effect than 7.5 and 10 kGy.

Studies on the antioxidant capacity of *Lycium* spp. by the ORAC method performed by Protti et al. (2017) presented results similar to those found in the ORAC test of the present study in goji-berries no processed by ionizing radiation. In contrast, studies as performed by Fanaro et al. (2014,2015) did not show any significant difference in antioxidant activity in the ORAC assay in irradiated samples of black tea and green tea treated with ionizing radiation up to 10 kGy.

3.3. Total phenolics and total flavonoid

The results total phenolics are expressed in Table 3 and demonstrated that the ionizing radiation processing of the goji-berry positively affected the total phenolic content, seen as the maximum irradiated values in relation to the non-irradiated samples (17.873 mg/g) the dose of 5.0 kGy having the highest phenolic content (26.969 mg/g). The same was observed in the total flavonoid assays, since, according to the results (Table 4), the total content of total flavonoids was relatively higher in the irradiated samples, while the non-irradiated samples showed a relatively lower phenolic content ($p < 0.05$). Samples irradiated at a dose of 10.0 kGy and non-irradiated samples, presented average reaching 2.825 and 1.542 mg/g, respectively.

Pereira et al. (2015) found an increase in total phenolic content of irradiated aromatic plants as compared to that of the non-irradiated at an irradiation dose at a maximum of 10.0 kGy, Fanaro et al., (2014) reported an increase in the phenolic content in 2.0 and 2.5 kGy treated *Cammellia sinensis*.

Futhermore, previous studies have shown that irradiation influenced phenolic and flavonoid content. The results of research conducted with samples of cinnamon, cloves, curcuma and soybeans treated with ionizing radiation demonstrated an increase in phenolic content in relation to non-irradiated samples (Variyar et al., 1998; Taheri et al., 2014).

Zúñiga et al. (2012) researched the irradiated extract of quillaia flowers presented phenolic content reduced only in the dose of 15.0 kGy. Also, in studies with edible flowers treated with the electron beam and gamma irradiation (up to 1.0 kGy), a higher concentration of

Table 1
Results of the DPPH radical- scavenging activity of *Lycium barbarum* fruits irradiated.

Sample (kGy)	EC_{50} values (mg/mL of extract)
Control	0.186 ± 0.01[a]
2.5	0.185 ± 0.01[a]
5.0	0.181 ± 0.01[a]
7.5	0.181 ± 0.01[a]
10.0	0.180 ± 0.01[a]

Values represent mean ± standard deviation. The superscript letter a in the same column mean present no have statistical difference ($p \leq 0.05$).

Table 2
Goji-berry irradiated results of the Oxygen Radical Absorvance Capacity assay – ORAC.

Sample (kGy)	Values (μmole TE/100 g)
Control	135.70 ± 4.08[a]
2.5	190.13 ± 9.16[b]
5.0	185.47 ± 7.25[b]
7.5	176.69 ± 12.86[c]
10.0	151.25 ± 14.12[d]

Values represent mean ± standard deviation. Micromole Trolox equivalents (μmole TE). The superscript letter a in the same column mean present no have statistical difference ($p \leq 0.05$).

Table 3
Goji-berry fruits results of Total Phenolics assay after electron beam irradiation.

Sample (kGy)	Values (mg GAE/100 g)
Control	17.873 ± 1.437[a]
2.5	19.799 ± 0.826[a]
5.0	26.969 ± 0.407[b]
7.5	25.013 ± 1.463[b]
10.0	25.310 ± 0.565[b]

Values represent mean ± standard deviation. Milligram gallic acid (mg GAE) equivalents (m). The superscript letter a in the same column mean present no have statistical difference ($p \leq 0.05$).

Table 4
Lycium barbarum fruit results of Total Flavonoid assay after electron beam irradiation.

Sample (kGy)	Values (mg/g of dry extract)
Control	1.452 ± 0.083[a]
2.5	2.471 ± 0.047[b]
5.0	2.533 ± 0.071[b]
7.5	2.729 ± 0.125[bc]
10.0	2.825 ± 0.033[c]

Values represent mean ± standard deviation. The superscript letter a in the same column mean present no have statistical difference ($p \leq 0.05$).

phenolic compounds was observed in the irradiated samples (Koike et al., 2015; Villavicencio et al., 2018).

4. Conclusion

In conclusion, irradiated and non-irradiated samples did not present a significant difference in antioxidant activity in the DPPH assay. On the other hand, our work showed that electron beam irradiation improved the total antioxidant activity of the samples in ORAC, as well as a total flavonoid and total phenolics assays.

From the standpoint of the functional properties, irradiation treatment significantly increase the presence of its phytochemicals after ionizing radiation processing in goji-berry.

That is the evidence that electron beam irradiation is a clean technology and a safe process with which is possible not only to maintain but also enhance the nutritional goji-berry quality.

Credit author statement

Flavio Thihara Rodrigues: Formal analysis, Writing - original draft, Preparation, Writing - review & editing, Preparation, Visualization, Resources Data curation, Investigation. Pamela Galo da Silva: Conceptualization, Investigation Conducting, Investigation, Resources. Bianca Guimarães Negrão: Data curation, Writing - review & editing. Amanda Cristina Ramos Koike: Investigation, Conducting, Writing - original draft, Preparation, Writing - review & editing, Resources, Data curation, Investigation. Severino Matias de Alencar: Preparation, Methodology, Writing - review & editing, Investigation. Jorge Mancini Filho: Methodology, Investigation. Anna Lucia Casañas Haasis Villavicencio: Conceptualization, Project administration, Supervision. Investigation, Resources, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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