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Effects of γ-radiation on microbial load and antioxidant proprieties in green tea irradiated with different water activities

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HIGHLIGHTS

- Higher the Aw, lower is the radiation dose to archive microbiology safety.
- The doses up to 10.0 kGy had no effect on antioxidant capacity in all Aw used.
- The recommended dose to irradiated green tea is 5.0 kGy.

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ABSTRACT

The aim of this paper is to study the effect of gamma radiation on green tea irradiated with different water activities. The green tea samples had their Aw adjusted to three values (0.93, 0.65, and 0.17) and were irradiated in ^{60}Co source at doses of 0, 1.0, 1.5, 2.0, 2.5, 5.0, 7.5, and 10.0 kGy. The methods used were: microbiology, total phenolic compounds quantification, antioxidant activity by ORAC, and quantification of the main antioxidants. It was observed that the greater the amount of free water present in the samples, lower was the dose to achieve microbiological control. Despite the irradiation with 5.0 kGy with high water activity has a small decrease in phenolic compounds and in some catechins content, this condition is recommended once was the dose to ensure microbiological safety without interfering in the main catechins and the antioxidant activity.

1. Introduction

Tea is regarded as a tasteful drink. However, the scientific community has recently re-discovered the therapeutic potential of this beverage (Pan et al., 2013). Green tea, consumed mainly in Japan, China and Korea, is produced when freshly harvested leaves of *Camellia sinensis* are subjected first to withering, and then pan-fried/steamed prior to rolling/shaping and drying (Santana-Rios qet al., 2001).

Epidemiologic studies have associated the consumption of green tea with low risk of several cancer developments (Sato and Myata, 2000; Toit et al., 2001) and its polyphenols have demonstrated to be an effective quimiopreventive agent (Cabrera et al., 2003; Gosslau and Chen, 2004). Other beneficial proprieties are related to to insulin-enhancing activity (Anderson and Polansky, 2002), antimicrobial, immunostimulatory, and anti-inflammatory capacities (Saito et al., 2006), protection against cardiovascular (Sano et al., 2004) and brain (Suzuki et al., 2004) diseases, and caries growth (Sakanaka et al., 1989). Nevertheless, if tea leaves are contaminated with pathogenic microorganisms, the infusion may represent a potential risk for health. Presence of fungal strains and aflatoxin are also reported in *C. sinensis* plants studies (Aquino et al., 2010; Bugno et al., 2006; Martins et al., 2001).

Ionizing radiation is one of the most effective means to disinfect foodstuffs. This treatment can inhibit living cellular division such as microorganisms and promote molecular structural modification (Farkas, 2006; Farkas and Mohácsi-Farkas, 2011; Villavicencio et al., 2007). Once absorbed by a biological material, gamma radiation has a direct and an indirect effect on the material that received this processing (Hansen and Shaffer, 2001). The primary mechanism, also called direct effect, is given mostly by the double-strand breaks of DNA molecule, causing the inactivation of that cell. This process is dominant when dry spores of microorganisms are irradiated (Tritsch, 2000).

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In biological systems, ionizing radiation causes the water ionization and excitation, leading to the formation of radiolytic products in a very short time (about 10^{-8} s). The interaction of ionizing radiation with the water molecule generates the so-called free radicals, a reaction commonly known as radiolysis (Riley, 1994; Hayes et al., 1995). These free radicals will interact with other cell components such as DNA, enzymes, macromolecules, vitamins, and antioxidant compounds present in the food matrix (Breen and Murphy, 1995; Monk et al., 1995). Due to the fact that the indirect effect of radiation is responsible for 70% of all radiation effects (Diehl, 2002), it is important in plant cells that have an abundant amount of water (Morehouse, 1998).

Even dry food such as wheat flour (13%), dehydrated vegetables (10%), and nuts (5%), have significant water content (World Health Organization, 1994). Therefore the aim of this study was to evaluate the effect of gamma radiation on microbiological load, antioxidant properties, and on mean compounds of green tea irradiated with different water activities.

2. Experimental

2.1. Samples

The green tea was donated by Herbarium Laboratório Botânico (Paraná, Brazil) and processed in triplicate.

2.2. Water activity (aw)

The samples were adjusted to three Aw values, a high Aw (0.931), an intermediary Aw (0.651), and a low Aw (0.170). To increase the Aw, the samples were placed in Petri dish left uncovered in a glass desiccator filled with distilled water until the disc level for 48 h/ ± 25 °C. To decrease the Aw, the samples were placed in petri dish and left uncovered in an incubator at 35 °C/48 h. The samples with intermediary Aw had their values unchanged and maintained at 25 °C (Fanaro et al., 2014).

The Aw were measured using an Aqualab 4TE Duo equipment (Decagon Devices Inc., USA) in duplicated. Then the samples were places into stomacher bags, sealed, and identified with their respective radiation doses. The period between the measuring and the irradiation process was less than 24 h. The Aw was not measured after the processing once some authors observed that the radiation process had no interference in Aw parameter (Chosdu et al., 1995; Mishra et al., 2006; Pezzutti et al., 2005).

The samples were weighted before the Aw adjustment, thus the amount of water gained or lost had no interference with the weight used in each experiment (the weight varied according to the test).

2.3. Irradiation

The samples were irradiated at room temperature (± 25 °C) in a ^{60}Co source Gammacell 220 (Nordion Ltd., Canada) at doses of 0, 2.5, 5.0, 7.5 and 10.0 kGy. Later, were added the doses of 1.0, 1.5 and 2.0 kGy. The dose rate during the period was between 2.16 and 1.43 kGy/h. Harwell Amber 3042 dosimeters were used to measure the radiation dose and the uncertainty dose was 2.25%. All the irradiation was performed in similar place, using a platform and a kind of capsule to make the samples always with the same height and length inside the machine.

Due to the course of time among the irradiation periods in this work, the procedures described above were also performed in these different periods (as replicate) to assure that the different dose rate had no influence in the results. So, the same techniques and the same reagents (always from the same manufacture) were used. As no statistical difference among the periods on the tests was found, the data of these replicates are not shown.

2.4. Microbiology assay

In a sterile stomacher bag, 10 g of sample ware mixed with 90 mL of sterile water (10^{-1} dilution) for 30 min. A total of 1 mL was transferred to tubes with 9 mL of sterile water and serial dilution was performed until the dilution 10^{-8}. The fraction of 0.1 mL of each tube, in triplicate, was placed in petri dish containing solid Dichloran Glycerol 18% agar (the powder from Acumedia, USA and the glycerol from Dinâmica, Brazil) and incubated at 25 ± 0.2 °C/5 days (Pitt et al., 1983).

The count was determined by colony forming units per gram of green tea plant (CFU/g). All the samples were processed in triplicate.

2.5. Artificial contamination

Due to the low fungal contamination found in donated samples, they were infected with strains of *Aspergillus* ssp. and *Rhizopus* ssp. provided by Toxigenic Fungus and Mycotoxin Laboratory from University of São Paulo.

A single platinum wire loop of each fungus dissolved in 10 mL of distilled water with a drop of Tween 20 was added to the samples in a polyethylene bag and mixed manually. The bags were incubated at 25 ± 0.2 °C/2 weeks and then the steps water activity, irradiation, and microbiology assay were repeated.

2.6. Soluble compounds extraction

An infusion was prepared with 5 g in 500 mL of distilled boiling water for 10 min with light agitation at the beginning, the middle, and the end of the period. A vacuum filtration was performed using regular filter paper. After cooling at room temperature (± 25 °C), the volume was adjusted to 500 mL with distilled water, aliquoted and stored at − 18 °C. The extraction was performed in triplicate.

2.7. Total phenolic compounds determination

The total phenolic compounds were determinate by the Folin-Ciocalteu reagent. In a 20 mL volumetric flask, 50 μL of extracts, 10 mL of distilled water and 1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, EUA) were added, mixed, and left in stand-by for 3 min. Then, 8 mL of saturated sodium carbonate (75 g/L) were added and the final volume was adjusted to 20 mL with distillated water. The flasks were incubated for 2 h/37 °C to the color development. The absorbance was read in a spectrophotometer of visible UV (UV-1601, Shimadzu) in quartz cuvette at wave length of 765 nm (Singleton and Rossi, 1965).

The quantification was performed through a calibration curve using Gallic acid (Sigma-Aldrich) as standard (range of 50–100 μg/mL, r^2=0,9974). The results are expressed as mg/100 mL of Gallic acid equivalent (mgGAE/100 mL). The analyses were performed in triplicate and a blank solution was made replacing the extract and standard by distillated water.

2.8. Antioxidant activity

The antioxidant activity was measured using the ORAC assay (Ou et al., 2001). Briefly, 50 μL of each extract diluted in distilled water (1:500 v/v) and 150 μL (93.54 nmol/L) of fluorescein (3′, 6′-dihydroxy-spiro[isobenzofuran-1[3H],9′[9H]-xanthen]-3-one), Sigma-Aldrich) were add in a 96-well polystyrene black microplate and incubated at 37 °C/15 min protect from light. Then, 50 μL

(221 mM) of AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride, Sigma-Aldrich) were added. The fluorescence was read in excitation and emission wavelength of 493 nm and 515 nm respectively in a microplate reader Spectramaz M5 equipment (Molecular Devices) at the beginning (time 0 min) and every minute for 60 min at 37 °C.

The Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich) was used as standard (20 μmol/L) and a phosphate buffer (75 mM) as blank. The ORAC value was determinate by the area under curve (AUC) and express in mmol of Trolox equivalent in 100 ml (mmolTE/100 mL) through the equations:

$$AUC = 1 + f1/f0 + f2/f0 + f3/f0 + f4/f0 + \ldots + f60/f0$$

where: $f0$ is the fluorescence read at time 0 min.

fn is the fluorescence read at time n min.

ORAC = 20 × sample dilution × (AUC sample − AUC blank)/(AUC standard − AUC blank)

The analyses were performed in triplicate and all reagents were prepared in phosphate buffer (75 mM, pH 7.4).

2.9. Green tea biocompounds

The green tea biocompounds separation, identification, and quantification were performed in a HPLC (Hewlett-Packard Infinity 1120 Series, Palo Alto, EUA) equipped with DAD detector. A Prodigy ODS_3 (250 mm × 4.60 mm × 5 μm) (Phenomenex Ltda, UK) column was used.

The gradient elution is present in Table 1 using water acidified with 0.5% of formic acid (A solution) and acetonitrile acidified with 0.5% of formic acid (B solution) (both from Merck, Germany). The flow rate was 1 mL/min and the column oven was set to 25 °C. The total run time was 45 min.

The samples were injected in duplicate in volume of 20 μL and monitored the wavelength of 270 and 370 nm. The compounds were identified through its retention time and absorption, in comparison of the same attributes obtained from the standards (all from Sigma-Aldrich): epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), galic acid, and caffeine. The quantification was performed through a 5-points calibration curve built to each compound and the results are expressed in mg/100 ml.

2.10. Statistical analysis

The results were expressed as mean ± standard deviation and the statistical analysis ANOVA and Tukey tests were performed using in both case the value of $p \leq 0.05$.

Table 1
Solvent gradient elution used, in percentage, to separate and identify the green tea biocompounds by HPLC/DAD.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
15	80	20
25	75	25
33	65	35
38	50	50
43	10	90
44	10	90
45	10	90

3. Results

3.1. Microbiology

By assessing the microbiological contamination of a green tea plant it was possible to observe the low fungal contamination (Table 2). However, when the Aw was increased, even during a relatively short time (48 h), the fungal contamination in the samples was higher than the other samples with lower Aw. That may be explained by the fact that the low aw values usually found in tea plants (0.65) contribute to decrease the cellular metabolism of microorganism. As Aw was increased, a slight rise on fungal metabolism was observed. Independently of Aw and initial contamination, the 2.5 kGy dose was sufficient to reduce the fungal contamination to not detected levels.

The World Health Organization (WHO) recommends that the fungal contamination for medicinal plants that will be used as hot infusion should be less than 10^5 CFU/g (World Health Organization, 1998). Although the values found in this paper are in agreement with WHO in all Aw, the literature has reported a high fungal biobirden in infusion plants, including the *C. sinensis* plant (Aquino et al., 2010; Martins et al., 2001). Even when the contamination were low, as in this paper, the major fungal species detected were mycotoxin-producer as *Aspergillus*, *Penicillium*, *Rhizopus*, and *Fusarium* genus (Řezáčová and Kubátová, 2005; Storari et al., 2012).

Due the low contamination and the initial radiation dose choices, it was not possible to observe whether the radiolysis had any influence on the decontamination process. Therefore, the green tea plant was artificially contaminated and the radiation doses of 1.0, 1.5, and 2.0 kGy were included, and the items 2.2, 2.3, and 2.4 were repeated. The new values are present in Table 3, where it is possible to verify that higher the Aw, the lower the radiation dose required to reduce microbiological contamination.

As found previously, the higher the initial Aw, the higher the initial contamination. Although the fungal load was higher in the green tea that had low and medium Aw, the radiation was more

Table 2
Fungal contamination, in CFU/g, of green tea plant irradiated with different radiation doses and Aw.

Aw	Doses (Kgy)				
	0	2.5	5.0	7.5	10.0
0.170	8.33×10^{2a}	ND	ND	ND	ND
0.651	23.0×10^{2a}	ND	ND	ND	ND
0.931	103.0×10^{2b}	ND	ND	ND	ND

ND means not detected.
[a,b]Different superscript latters in the same column mean statistical difference ($p \leq 0.05$).

Table 3
Artificially fungal contamination by *Aspergillus* ssp. and *Rhizopus* ssp., in CFU/g, in green tea plant irradiated with different radiation doses and Aw.

Aw	Doses (kGy)							
	0	1.0	1.5	2.0	2.5	5.0	7.5	10.0
0.170	1.1×10^{8a}	3.3×10^{5a}	4.0×10^{4a}	2.7×10^{3a}	2.7×10^{2}	ND	ND	ND
0.651	1.2×10^{8a}	5.2×10^{4b}	3.7×10^{3b}	1.6×10^{2b}	ND	ND	ND	ND
0.931	1.8×10^{8b}	2.0×10^{4b}	1.7×10^{2b}	ND	ND	ND	ND	ND

ND means not detected.
[a,b]Different superscript latters in the same column mean statistical difference ($p \leq 0.05$).

effective to decrease the contamination in this condition (high Aw). At 1.0 kGy the fungal count was more than 10 times lower in high Aw sample when compared to the low Aw sample, and the radiation dose to reduce completely the fungal contamination at high Aw was more the half, comparing the low Aw with high Aw and was the half when comparing the medium Aw with high Aw. Similar effects were found in black tea irradiated at same conditions (Fanaro et al., 2014).

The results that demonstrated that 5.0 kGy was the minimum dose with no fungal growth in the sample with low Aw sample is in agreement with literature. Katusin-Razem et al. (2001) reported that the range of doses from 5.0 to 8.0 kGy (Aw was not measured) is sufficient to decontaminate several dried vegetal materials when the fungal burden is higher than 10^4 CFU/g. Mishra et al. (2006) verified that at least 5.0 kGy was required to eliminate the fungal development, specially the *Aspergillus* genus in *C. sinensis* plant with the Aw of approximately 0.39 and initial fungi contamination of 10^4 CFU/g. The fungal growth was not observed even when the storage conditions were in a 99.9% moisture for 11 days.

Aquino et al. (2010) observed that *C. sinensis* with Aw of 0.58 irradiated at 5.0 kGy had a bioburden decreased to not detectable levels in 17 of 20 samples, and in only one sample, that had fungal growth, remained contaminated after 30 days of storage with a total of 5×10^2 CFU/g. Therefore, the authors recommended the dose of 10.0 kGy, because it was the radiation dose that had no fungal presence in all samples after the storage period.

The fact that irradiated tea with high Aw requires lower radiation dose to decrease the contamination is not only related to the effect of radiolysis. Following the microorganisms growth curve, there are 4 phases that characterize their development. The Lag phase (no cell number variation), the Log phase (exponential growth), the Stationary phase (decreasing of growth velocity), and the Cell death phase.

As the tea Aw is usually low, most microorganisms are either in latency condition or with low metabolism or in spore form. By increasing Aw, the cells increase their metabolism, reaching the Log phase. At this phase, due to the high reproduction and metabolism speed, the cells are much more sensible to radiation (Tortora et al., 2009). A synergism between this phenomenon and the radiolysis could explain the lower radiation dose required to decrease fungal contamination in plants with high Aw.

3.2. Total phenolic compounds

Unlike of fungal contamination the total phenolic compounds data show the radiolysis effect on green tea, once when is irradiated with low Aw, no statistical difference ($p > 0.05$) among the doses were observed (Table 4), as well as this plant when irradiated with medium Aw, where only de dose of 5.0 kGy were different from other doses. However, when the green tea with high Aw was irradiated, more statistical differences among doses were observed.

The higher the Aw, the lower the phenolic compounds detected. Even the dose of 1.0 kGy, in which the greatest amounts of phenolic compounds were quantified, the values were lower than the amount found in medium and low Aw, except the control dose. In comparison to the other doses, the radiation decreased the amount of phenolics at the doses of 2.0, 2.5, 5.0, and 7.5 kGy. However, no difference ($p > 0.05$) was observed between the control and 10.0 kGy. Concerning the samples with medium aw, the difference presented at 5.0 kGy cannot be considering negative, once this dose increased the detected phenolic compounds.

By comparing the different Aw at the same radiation dose it was observed that the control dose had the same values at the three water activities. At 2.5 and 7.5 kGy doses, the lower was the Aw, higher was the amount detected. Higher values of phenolics were observed at 7.5 kGy with low aw. The dose of 5.0 kGy had the largest difference among the Aw (16.3 mg).

At the doses of 1.0 and 10.0 kGy, the plant with high and medium Aw had lower amount of phenolics than the tea irradiated with low Aw. At doses of 1.5, 2.0, and 5.0 kGy, lower values were detected in the samples with low aw in comparison to samples with medium and high water activities.

Regarding the doses observed in the microbiology, at the dose of 2.0 kGy (dose that decreased the fungal load to not detectable levels in samples with high aw) the total phenolic amount was lower than the dose of 2.5 kGy with medium Aw and lower than 5.0 kGy with low Aw, demonstrating that the radical formed by the radiolysis interact in microorganism and in phenolic compounds as well.

Turkmen et al. (2006) verified lower values (30.5 mg) while Atoui et al. (2005) reported higher amounts (352 mg) than this paper. This difference among authors can be explained due the fact that several compounds in plants vary according to the age, area/place of cultivations, genetic characteristics (Cardozo et al., 2007; Dartora et al., 2011), and the kind and time of the extraction (Ramalho et al., 2013).

3.3. Antioxidant activity

Although the results of phenolic compounds presented variations according to the doses the ORAC assay showed that the radiation up to 10.0 kG, even at different Aw has apparently no interference with antioxidant capacity of green tea (Table 5). However, at doses of 1.5 and 2.0 kGy as higher was the Aw, lower was the antioxidant activity. It was not possible to explain why this

Table 4
Amount of total phenolic compounds, in mgGAE/100 mL of green tea, from *C. sinensis* irradiated with different radiation doses and Aw.

Doses (kGy)	0.170	0.651	0.931
0	93.7 ± 7.8^{ax}	90.6 ± 1.2^{ax}	89.5 ± 0.5^{ax}
1.0	97.2 ± 0.5^{ax}	92.5 ± 0.2^{ay}	91.9 ± 0.5^{by}
1.5	95.9 ± 0.8^{ax}	94.3 ± 2.9^{abx}	88.2 ± 0.3^{ay}
2.0	98.4 ± 0.6^{ax}	96.1 ± 2.1^{abx}	83.4 ± 0.1^{cy}
2.5	95.7 ± 0.2^{ax}	91.7 ± 1.1^{ay}	80.7 ± 1.7^{dz}
5.0	99.7 ± 1.7^{ax}	98.9 ± 4.5^{bx}	83.4 ± 1.3^{cy}
7.5	100.3 ± 1.3^{ax}	96.4 ± 1.9^{aby}	84.8 ± 0.2^{cz}
10.0	99.9 ± 1.9^{ax}	93.3 ± 4.0^{aby}	89.8 ± 0.2^{ay}

Values represent mean ± standard deviation.
[a,b,c,d] Different superscript latters in the same column mean statistical difference ($p \leq 0.05$).
[x,y,z] Different superscript latters in the same line mean statistical difference ($p \leq 0.05$).

Table 5
ORAC assay values, in mmolTE/100 mL of green tea, from *C. sinensis* plant irradiated with different radiation doses and Aw.

Doses (kGy)	0.170	0.651	0.931
0	21.0 ± 2.3^{ax}	21.3 ± 2.1^{abcdex}	21.1 ± 2.1^{ax}
1.0	22.6 ± 0.88^{ax}	22.4 ± 0.9^{adex}	21.0 ± 3.6^{ax}
1.5	22.5 ± 1.1^{ax}	18.8 ± 0.9^{bcdy}	19.4 ± 1.8^{ay}
2.0	22.0 ± 1.7^{ax}	18.3 ± 1.2^{bcy}	19.3 ± 0.9^{ay}
2.5	21.4 ± 2.9^{ax}	21.7 ± 1.9^{adex}	21.7 ± 2.3^{ax}
5.0	21.3 ± 2.8^{ax}	22.5 ± 1.3^{adex}	21.0 ± 2.3^{ax}
7.5	22.5 ± 0.9^{ax}	20.4 ± 1.3^{abcdex}	20.1 ± 3.4^{ax}
10.0	22.0 ± 1.4^{ax}	20.8 ± 0.6^{abcdex}	21.0 ± 3.0^{ax}

Values represent mean ± standard deviation.
[a,b,c,d,e] Different superscript latters in the same column mean statistical difference ($p \leq 0.05$).
[x,y,z] Different superscript latters in the same line mean statistical difference ($p \leq 0.05$).

phenomenon occurred; nevertheless, it was not caused by the radiolysis, once except these two doses, the antioxidant activity had no variation at all water activities and radiation doses used.

Fanaro et al. (2014) observed no difference in ORAC values in black tea irradiated at the same doses and with a very similar water activities as reported in this paper. Kumar et al. (2010) working with several phytotherapics and Mishra et al. (2006) studding *C. sinensis*, demonstrated that the radiation up to 10.0 kGy has no influence on antioxidant activity by DPPH assay.

3.4. Identification and quantification of biocompounds

Due to the results found in this paper from microbiology, total phenolic compounds, and antioxidant activity assays, the main green tea flavonoids and the compounds Gallic acid and caffeine were identified and quantified only at 5.0 kGy, which was the minimum radiation dose to ensure the microbiological safety at all Aw, and on 10.0 kGy which is the dose usually recommended for this kind of plant by several countries and authors. Also, in these doses, no flavor change was observed (Fanaro et al., 2011), which is a very important parameter in this kind of beverage.

According to the results found in total phenolic compounds assay, the radiolysis demonstrated to have influence on the main green tea flavonoids, because greater amounts of flavonoids were observed when this plant was irradiated at low aw and smaller amounts when it was irradiated at high aw. These results were observed in the majority of compounds analyzed (Table 6).

The increase of Gallic acid can be explained by the degradation of gallate compounds. It was observed that with high Aw, when the radiation dose was increased, higher was the amount of Gallic acid compound, although this difference was not significant; however at 10.0 kGy the difference was statistical significant, where higher was the Aw, higher was the Gallic acid identified.

Concerning the epicatechin, the green tea plant when irradiated with 5.0 kGy with Aw above of 0.6 has the amount decreased, whereas the irradiation with low Aw increases this compound. Besides that, when irradiated with high Aw, as the dose was increased, lower was the amount detect. A similar effect can be observed with the epigallocatechin.

The epicatechin gallate analysis showed that with low Aw at the dose of 5.0 kGy there was an increased in the amount of this compound. Nevertheless, at 10.0 kGy with medium and high Aw the amount of the compound. The amount of epigallocatechin gallate decreased when the *C. sinensis* plant was irradiated at 5.0 kGy with medium Aw and at 10.0 kGy with high Aw.

The caffeine compound had no influence in both direct and indirect effect of radiation in the different doses and Aw used, differently found previously in black tea (Fanaro et al., 2014), which the higher was the radiation dose, the grater the amount of caffeine detected. It could be explained by although both teas became from the same plant, the kind of manufacturing influences directly on the plant structures.

The literature also reports the presence of other kinds of phenolic compounds in this beverage; however, the compounds catechins, catechins gallate, quercetin, myricetin, and kaempferol were not detected.

So, the use of radiation process is highly recommended to green tea plant, once the dose up to 5.0 kGy even decrease the content of some kinds of catechins when irradiated with high Aw, ensure a microbial safety product without compromise the antioxidant activity which is one of mainly characteristic of this drink. Furthermore, the catechins decrease is very slow and is insignificant when the tea is consumed regularly.

The amount of catechins and caffeine compounds in green tea were higher than the amounts reported by Reto et al. (2007) in their nine kinds of green tea from Portugal, and El-Shahawi et al. (2012) in their 29 samples from a Saudi Arabia market. On the

Table 6
Amount of total main catechins, Gallic acid and caffeine, in mg/100 mL of green tea, identified in ***C. sinensis*** irradiated with different radiation doses and Aw.

Compounds	Doses (kGy)	Aw		
		0.170	**0.651**	**0.931**
Gallic acid	**0**	3.41 ± 0.18^{ax}	3.42 ± 0.05^{ax}	3.44 ± 0.37^{ax}
	5.0	3.41 ± 0.09^{ax}	3.60 ± 0.18^{ax}	3.65 ± 0.51^{ax}
	10.0	3.30 ± 0.09^{ax}	3.45 ± 0.05^{ay}	3.77 ± 0.22^{az}
Epicatechin (EC)	**0**	11.8 ± 0.1^{ax}	11.9 ± 0.1^{ax}	11.8 ± 0.1^{ax}
	5.0	12.1 ± 0.2^{bx}	11.5 ± 0.1^{by}	11.5 ± 0.1^{by}
	10.0	12.1 ± 0.3^{bx}	11.7 ± 0.3^{aby}	11.4 ± 0.1^{by}
Epicatechin gallate (ECG)	**0**	18.4 ± 0.1^{ax}	18.3 ± 0.1^{ax}	18.4 ± 0.3^{ax}
	5.0	19.0 ± 0.1^{bx}	18.3 ± 0.1^{ay}	18.3 ± 0.2^{ay}
	10.0	18.5 ± 0.5^{ax}	17.9 ± 0.8^{ax}	17.9 ± 0.1^{bx}
Epigallocatechin (EGC)	**0**	62.9 ± 1.0^{ax}	62.2 ± 0.6^{ax}	62.7 ± 0.7^{ax}
	5.0	61.8 ± 0.7^{ax}	60.2 ± 0.3^{by}	60.5 ± 1.2^{by}
	10.0	61.6 ± 0.8^{ax}	60.7 ± 0.9^{bx}	58.9 ± 0.7^{cy}
Epigallocatechin gallate (EGCG)	**0**	43.1 ± 0.9^{ax}	42.5 ± 0.1^{ax}	42.8 ± 0.6^{ax}
	5.0	43.0 ± 0.7^{ax}	41.6 ± 0.6^{ay}	42.8 ± 0.6^{ax}
	10.0	42.5 ± 1.4^{ax}	42.5 ± 1.5^{ax}	42.0 ± 0.3^{bx}
Caffeine	**0**	38.5 ± 0.9^{ax}	37.8 ± 0.2^{ax}	38.5 ± 0.5^{ax}
	5.0	39.3 ± 0.9^{ax}	37.6 ± 0.9^{ax}	38.4 ± 1.5^{ax}
	10.0	38.4 ± 0.9^{ax}	37.4 ± 0.8^{ax}	37.2 ± 0.4^{ax}

Values represent mean $\pm$ standard deviation.
^{a,b,c} Different superscript latters in the same column mean statistical difference ($p \leq 0.05$).
^{x,y,z} Different superscript latters in the same line mean statistical difference ($p \leq 0.05$).

other hand, in four samples from China, Wu et al. (2012) identified a higher amount of EGCG, but lower levels of EGC and EC, and similar values of ECG that found in this work. These amount variations were explained in item 3.2.

Despite the radiation decreased the main green tea compounds, mainly when the plant was irradiated with high water activity, this reduction even was statistical, could be considerate insignificant once the highest statistical difference was found in the EGC compound irradiated at high Aw between the control and 10.0 kGy (3.8 mg/100 ml of difference) doses, and the smaller statistical difference was presented by EC compound (0.3 mg/100 ml). Besides that, the data from antioxidant activity demonstrated that there was no decreasing of antioxidant activity among the treatments used. Additionally, it does not mean that tea irradiated at high aw will bring less health benefits for people who drink it. Other factors such as absorption, metabolizing and excretion influence this beverage ingestion

Animal studies showed that the EGCG is mainly excreted through the bile, while the EGC and EC are excreted in the urine and bile, indicating that the catechins are quickly and extensive metabolized. As the EGCG was not detected in urine, 90% from total of EC and EGC were excreted in urine for 8 h and no compound was detect in the organism after 24 h (Yang et al., 1998).

Higdon and Frei (2003) compered the pharmacokinetic of equimolar doses of pure EGC, ECG, and EGCG in 10 human health volunteers. The average peaks concentrations in plasma after a single dose of 1.5 mmol were 5 μmol/L to EGC, 3.1 μmol/L to ECG, and 1.3 μmol/L to EGCG. The plasmatic values of EGC and EGCG were back to base levels while the plasma concentration of ECG remained high after 24 h. Therefore, the maintenance of high plasmatic concentration of catechins requires a usual ingestion over time and not only the consumption of tea with high levels of catechins (Scalbert et al., 2002).

4. Conclusion

The irradiation with high Aw (0.931) increases the radiation effects on fungal decontamination, requiring a lower dose to microbiological control when comparing to a normal Aw and low Aw, without interfering in the major flavonoids content and antioxidant activity at doses up to 5.0 kGy.

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References

Anderson, R.A., Polansky, M.M., 2002. Tea enhances insulin activity. J. Agric. Food Chem. 50, 7182–7186.
Aquino, S., Gonçalez, E., Rossi, M.H., Nogueira, J.H.C., Reis, T.A., Correa, B., 2010. Evaluation of fungal burden and aflatoxin presence in packed medicinal plants treated by gamma radiation. J. Food Prot. 73, 932–937.
Atoui, A.K., Mansouri, A., Boskou, G., Kefalas, P., 2005. Tea and herbal infusions: their antioxidant activity and phenolic profile. Food Chem. 89, 27–36.
Breen, A.P., Murphy, J.A., 1995. Reactions of oxyl radicals with DNA. Free Radic. Biol. Med. 18, 1033–1077.
Bugno, A., Almodovar, A.A.B., Pereira, T.C., Pinto, T.J.A., Sabino, M., 2006. Occurrence of toxigenic fungi in herbal drugs. Braz. J. Microbiol. 37, 47–51.
Cabrera, C., Gimenez, R., López, M.C., 2003. Determination of tea components with antioxidant activity. J. Agric. Food Chem. 51, 4427–4435.
Cardozo Jr., E.L., Ferrarese-Filho, O., Cardozo Filho, L., Ferrarese, M.L.L., Donaduzzi, C.M., Sturion, J.A., 2007. Methylxanthines and phenolic compounds in mate (*Ilex paraguariensis* St. Hil.) progenies grown in Brazil. J. Food Comp. Anal. 20, 553–558.
Chosdu, R., Erizal, Iriawan, T., Hilmy, N., 1995. The effect of gamma irradiation on curcumin component of *Curcuma domestica*. Radiat. Phys. Chem. 46, 663–667.
Dartora, N., Souza, L.M., Santana-Filho, A.P., Iacomini, M., Valduga, A.T., Gorin, P.A.J., Sassaki, G.L., 2011. UPLC-PDA-MS evaluation of bioactive compounds from leaves of *Ilex paraguariensis* with different growth conditions, treatments and ageing. Food Chem. 129, 1453–1461.
Diehl, J.F., 2002. Food irradiation – past, present and future. Radiat. Phys. Chem. 63, 211–215.
El-Shahawi, M.S., Hamza, A., Bahaffi, S.O., Al-Sibaai, A.A., Abduljabbar, T.N., 2012. Analysis of some selected catechins and caffeine in green tea by high performance liquid chromatography. Food Chem. 134, 2268–2275.
Fanaro, G.B., Duarte, R.C., Araújo, M.M., Purgatto, E., Villavicencio, A.L.C.H., 2011. Evaluationof γ-radiation on green tea odor volatiles. Radiat. Phys. Chem. 80, 85–88.
Fanaro, G.B., Hassimotto, N.M.A., Bastos, D.H.M, Villavicencio, A.L.C.H., 2014. Effects of γ-radiation on microbial load and antioxidant proprieties in black tea irradiated with different water activities. Radiat. Phys. Chem. 97, 217–222.
Farkas, J., 2006. Irradiation for better foods. Trends Food Sci. Technol. 17, 148–152.
Farkas, J., Mohácsi-Farkas, C., 2011. History and future of food irradiation. Trends Food Sci. Technol. 22, 121–128.
Gosslau, A., Chen, K.Y., 2004. Nutraceuticals, apoptosis, and disease prevention. Nutrition 20, 95–101.
Hansen, J.M., Shaffer, H.L., 2001. Sterilization and preservation by radiation sterilization. In: Block, S.S. (Ed.), Disinfection Sterilization and Preservation, fifth ed. Lippincott Willians & Wilkins, Philadelphia, pp. 729–746.
Hayes, D.J., Murano, E.A., Murano, P.S., Olson, D.G., Sapp, S.G., 1995. Food Irradiation: A Sourcebook, first ed. Iowa State University Press, Ames, Iowa.
Higdon, J.V., Frei, B., 2003. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit. Rev. Food Sci. Nutr. 43, 89–143.
Katusin-Razem, B., Novak, B., Razem, D., 2001. Microbiological decontamination of botanical raw materials and corresponding pharmaceutical products by irradiation. Radiat. Phys. Chem. 62, 261–275.
Kumar, S., Gautam, S., Powar, S., Sharma, A., 2010. Microbial decontamination of medicinally important herbals using gamma radiation and their biochemical characterization. Food Chem. 119, 328–335.
Martins, H.M., Martins, M.L., Dias, M.I., Bernardo, F., 2001. Evaluation of microbiological quality of medicinal plants used in natural infusions. Int. J. Food Microbiol. 68, 149–153.
Mishra, B.B., Gautam, S., Sharma, A., 2006. Microbial decontamination of tea (*Camellia sinensis*) by gamma radiation. J. Food Sci. 71, M151–M156.
Monk, J.D., Beuchat, L.R., Doyle, M.P., 1995. Irradiation inactivation of food-borne microorganisms. J. Food Prot. 58, 197–208.
Morehouse, K.M., 1998. Food irradiation-US regulatory considerations. Radiat. Phys. Chem. 63, 281–284.
Ou, B., Hampsch-Woodill, M., Prior, R., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent. J. Agric. Food Chem. 49, 4619–4626.
Pan, M.H., Lai, C.S., Wang, H., Loc, C.Y., Hod, C.T., Li, S., 2013. Black tea in chemoprevention of cancer and other human diseases. Food Sci. Hum. Wellness 2, 12–21.
Pezzutti, A., Matzkin, M.R., Croci, C.A., 2005. Gamma irradiation improved the quality of onion flakes used by argentine consumers. J. Food Process. Preserv. 29, 120–131.
Pitt, J.I., Hocking, A.D., Glenn, D.R., 1983. An improved medium for the detection of *Aspergillus flavus* and *Aspergillus parasiticus*. J. Appl. Bacteriol. 54, 109–114.
Ramalho, S.A., Nigam, N., Oliveira, G.B., Oliveira, P.A., Silva, T.O.M., Santos, A.G.P., Narain, N., 2013. Effect of infusion time on phenolic compounds and caffeine content in black tea. Food Res. Int. 51, 155–161.
Reto, M., Figueira, M.F., Filipe, H.M., Almeida, C.M.M., 2007. Chemical composition of green tea (*Camellia sinensis*) infusions commercialized in Portugal. Plant Foods Hum. Nutr. 62, 139–144.
Řezáčová, V., Kubátová, A., 2005. Saprobic microfungi in tea based on *Camellia sinensis* and on other dried herbs. Czech Mycol. 57, 79–89.
Riley, P.A., 1994. Free radicals in biology: oxidative stress and the effects of ionizing radiation. Int. J. Radiat. Biol. 65, 27–33.
Saito, S.T., Welzel, A., Suyenaga, E.S., Bueno, F., 2006. A method for fast determination of epigallocatechin gallate (EGCG), epicatechin (EC), catechin (C) and caffeine (CAF) in green tea using HPLC. Ciênc. Technol. Aliment. 26, 394–400.
Sakanaka, S., Kim, M.J., Yamamoto, T., 1989. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a carciogenic bacterium. Agric. Biol. Chem. 53, 2307–2311.
Sano, J., Inami, S., Seimiya, K., Ohba, T., Sakai, S., Takano, T., Mizuno, K., 2004. Effects of green tea intake on the development of coronary artery disease. Cir. J. 68, 65–670.
Santana-Rios, G., Orner, G.A., Amantana, A., Provost, C., Wu, S.Y., Dashwood, R.H., 2001. Potent antimutagenic activity of white tea in comparison with green tea in the *Salmonella* assay. Mutat. Res. 495, 61–74.
Sato, T., Myata, G., 2000. The nutraceutical benefit, Part I: green tea. Nutrition 16, 315–317.
Scalbert, A., Morand, C., Manach, C., Rémésy, C., 2002. Absorption and metabolism of polyphenols in the gut and impact on health. Biomed. Pharmacother. 56, 276–282.
Singleton, V.L., Rossi Jr, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.
Storari, M., Dennert, A.G., Bigler, L., Gessler, C., Broggini, G.A.L., 2012. Isolation of mycotoxins producing black aspergilli in herbal teas available on the Swiss market. Food Control 26, 157–161.

Suzuki, M., Tabuchi, M., Ikeda, M., Umegaki, K., Tomita, T., 2004. Protective effects of green tea catechins on cerebral ischemic damage. Med. Sci. Monit. 10, 166–174.
Toit, R., Volsteedt, Y., Apostolides, Z., 2001. Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. Toxicology 166, 63–69.
Tortora, G.J., Funke, B.R., Case, C.L., 2009. Microbiology: A introduction, tenth ed. Benjamin-Cummings, San Francisco.
Tritsch, G.L., 2000. Food iradiation. Nutrition 16, 698–701.
Turkmen, N., Sari, F., Velioglu, Y.S., 2006. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chem. 99, 835–841.
Villavicencio, A.L.C.H., Fanaro, G.B., Araújo, M.M., Aquino, S., Silva, P.V., Mancini-Filho, J., 2007. Detection of *Phakopsora pachyrhizi* by polymerase chain reaction (PCR) and use of germination test and DNA comet assay after e-beam processing in soybean. Radiat. Phys. Chem. 76, 1878–1881.
World Health Organization, 1994. Safety and Nutritional Adequacy of Irradiated Food. World Health Organization, Geneva, Switzerland.
World Health Organization, 1998. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva, Switzerland p. 122.
Wu, C., Xu, H., Héritier, J., Andlauer, W., 2012. Determination of catechins and flavonol glycosides in Chinese tea varieties. Food Chem. 132, 144–149.
Yang, C.S., Chen, L., Lee, M.J., Balentine, D., Kuo, M., Schantz, S.P., 1998. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol. Biomark. Prev. 7, 351–354.