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# Effects of γ-radiation on microbial load and antioxidant proprieties in black 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 black tea is 5.0 kGy.

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## ABSTRACT

The aim of this paper is to study the effect of gamma radiation on black tea irradiated with different water activities. The black tea samples had their Aw adjusted to three values (0.92, 0.65, and 0.18) 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. Regardless the water activity used, there was no difference in content of the phenolic compounds and at the mainly theaflavins, as well in the antioxidant activity at doses up to 5.0 kGy.



## 1. Introduction

Black tea, made from leaves of *Camellia sinensis* (L.) O. Kuntze, is the most widely consumed non-alcoholic drink (Owuor et al., 2008). India is a major producer and global exporter of this beverage (Borse et al., 2002). After the tea leaves are plucked, a number of processing stages, such as withering, pre-conditioning, cut-tear-curl operation, fermentation (enzymatic processes), and drying are involved in the production of black tea. Out of these processing stages, the fermentation is one of the critical operations. During this process, tea leaves change color from green to coppery brown or black and the grassy odor is transformed into floral (Bhattacharyya et al., 2007).

Tea is considered a tasteful drink and scientific community has recently re-discovered the therapeutic potential of this beverage (Pan et al., 2013). Its consumption has increased around the world because of its health benefits. Several studies indicate that tea and its catechins prevent and retard the development or progression of some diseases (Adhikary et al., 2011; Aneja et al., 2004; Khan and Mukhtar, 2007; Yoshino et al., 2010). Epidemiological studies have shown an inverse correlation between the risk of coronary heart disease and black tea consumption (Bahorun et al., 2012; Duffy et al., 2001). 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; Thomas et al., 2008; 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 which the radiation destroys microorganisms, also called direct effect, is given mostly

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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).

The indirect effect, responsible for 70% of all radiation effects, is caused by the interaction of ionizing radiation with the water molecule, generating the so-called free radicals. In biological systems, ionizing radiation causes the water ionization and excitation, leading to the formation of radiolytic products such as aqueous or hydrated electron ($e_{aq}^-$), ionized water ($H_2O^+$), hydroperoxyl radical ($HO_2^\bullet$), hydroxyl radical ($^\bullet OH$), hydrogen radical ($H^\bullet$), and hydrogen peroxide ($H_2O_2$) in a very short time (about $10^{-8}$ s) (Breen and Murphy, 1995; Diehl, 2002; Riley, 1994).

These free radicals will interact with other cell components such as DNA, enzymes, macromolecules, vitamins, and antioxidant compounds present in the food matrix. The indirect effect is important in plant cells that have an abundant quantity of water (Hayes et al., 1995; Monk et al., 1995; Morehouse, 1998). Even dry food has a significant water content, such as wheat flour (13%), dehydrated vegetables (10%), nuts (5%), among others (WHO, 1994). Therefore the aim of this study was to evaluate the effect of gamma radiation on microbiological load, antioxidant proprieties, and on mean compounds of black tea irradiated with different water activities.

## 2. Material and methods

### 2.1. Samples

The black tea was donated by Leão Junior S.A. (Paraná, Brazil) and processed in triplicate.

### 2.2. Water activity (Aw)

The samples were adjusted to three Aw values, a high Aw (0.924), an intermediary Aw (0.651), and a low Aw (0.183). 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.

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 depending of 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.

### 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 determinate by colony forming units per gram of black 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 normal 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 Jr, 1965).

The quantification was performed through a calibration curve using Gallic acid (Sigma-Aldrich) as standard (range of 50 to 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[3*H*],9′[9*H*]-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:

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

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. Black tea biocompounds

The black 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 the absorption, in comparison of the same attributes obtained from the standards (all from Sigma-Aldrich): theaflavins (mix of theaflavin, theaflavin-3 gallate, theaflavin-3′galatte, and theaflavine-3,3′ digallatte), 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 $p < 0.05$.

## 3. Results

### 3.1. Microbiology

By assessing the black tea plant microbiological contamination it was possible to verify the low fungal contamination (Table 2).

**Table 1**
Solvent gradient elution used, in percentage, to separate and identify the black 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 |

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

| Aw | Doses (kGy) | | | | |
|---|---|---|---|---|---|
| | 0 | 2.5 | 5.0 | 7.5 | 10.0 |
| **0.183** | $7.0 \times 10^{2a}$ | ND | ND | ND | ND |
| **0.651** | $10.3 \times 10^{2a}$ | ND | ND | ND | ND |
| **0.924** | $90.0 \times 10^{2b}$ | ND | ND | ND | ND |

ND means not detected.

[a,b] Different superscript letters in the same column mean statistical difference ($p \leq 0.05$).

However, when the Aw was increased, even in a relatively short time (48 h), the fungal contamination was higher than the other samples with lower Aw, due the fact that the low Aw values usually found in tea plants (0.65) contribute to decreasing the microorganism cellular metabolism. 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 recommends that medicinal plants that will be used as hot infusion, the fungal contamination should be less than $10^5$CFU/g (WHO, 1998). Despite the values found in this paper are in agreement with WHO in all Aw, papers described a high fungal biobirden in infusion plants, including the *C. sinensis* plant (Aquino et al., 2010; Bugno et al., 2006), and even those that the contamination were low, as in this paper, the major fungal detected were mycotoxin producer as *Aspergillus*, *Penicillium*, *Rhizopus*, and *Fusarium* genus (Řezáčová and Kubátová, 2005; Storari et al., 2012).

So, due the low contamination and the initial radiation dose choices, it was not possible to verify whether the radiolysis has any influence on the decontamination process. Therefore, the black tea plant was artificially contaminated and the radiation doses of 1.0, 1.5, and 2.0 kGy were included, and the microbiology assay was repeated. The new values are present in Table 3, where it is possible to verify that higher the Aw, lower the radiation dose required to reduce microbiological contamination.

As found previously, higher the initial Aw, higher the initial contamination. Although the fungal load was almost 10 times greater than in the black tea with low Aw, the radiation was more effective to decrease the contamination in this condition. At 1.0 kGy the fungal count was 10 times lower in high Aw when compared to the low Aw, and the radiation dose to reduce completely the fungal contamination was more the half, when compared both Aw.

The results demonstrating that the dose of 5.0 kGy was the minimum dose with no fungal growth in the low Aw sample is in agreement wiht literature. Katusin-Razem et al. (2001) reported that the range of 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 needed to eliminate the fungal development, specially the *Aspergillus* genus in *C. sinensis* with the Aw approximately of 0.39 and initial fungi contamination of $10^4$CFU/g, and even when stored in a 99.9% moisture for 11 days, no growth was found.

Aquino et al. (2010) observed that *C. sinensis* with 0.58 of Aw 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, continued contaminated after 30 days of storage with a total of $5 \times 10^2$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.

**Table 3**
Artificially fungal contamination by *Aspergillus* ssp. and *Rhizopus* ssp., in CFU/g, in black 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 |
| **0.183** | $3.7 \times 10^{7a}$ | $77.0 \times 10^{4a}$ | $200.0 \times 10^{2a}$ | $24.0 \times 10^{2a}$ | $3.7 \times 10^{2}$ | ND | ND | ND |
| **0.651** | $12.0 \times 10^{7b}$ | $6.2 \times 10^{4b}$ | $16.0 \times 10^{2b}$ | $9.3 \times 10^{2b}$ | ND | ND | ND | ND |
| **0.924** | $14.0 \times 10^{7b}$ | $6.1 \times 10^{4b}$ | $8.3 \times 10^{2c}$ | ND | ND | ND | ND | ND |

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

Thomas et al. (2008) described that two kinds of black tea irradiated at 7.0 kGy had their contamination reduced to not detectable levels; however, no lower radiation dose was used and the Aw was not measured.

The fact that irradiated tea with high Aw need lower radiation dose to decrease the contamination is not only related on the effect of radiolysis. Following the microorganisms growth curve, there are 4 phases that characterize their development. The Lag phase (no cell number variation), Log phase (exponential growth), Stationary phase (decreasing of growth velocity), and the Cell death phase. As the tea Aw usually is low, most of microorganisms are either in latency condition or with low metabolism or in spore form. With the increase of Aw, the cells increased their metabolism, reaching the Log phase, and in this phase, due to the high reproduction and metabolism velocity, the cells are much more sensible to radiation (Tortora et al., 2009). The synergism of this phenomenon with the radiolysis makes that the radiation dose be lower to decrease fungal contamination in plants with high Aw.

### 3.2. Total phenolic compounds

In general, when the Aw is increased, the amount of phenolic compounds detected is lower (Table 4). Concerning the samples irradiated with low Aw, it was observed that the greater the radiation dose (up to 2.0 kGy), the greater the amount of phenolic compound. However, when the radiation dose was increased, no statistical significance among the doses lower and higher than 2.0 kGy was found, except at 10.0 kGy. At 10.0 kGy the amount was statistically the same as the control sample.

The greatest amount of phenolic compounds was found in low Aw. Also, the largest difference among the radiation doses was observed in this condition. The most significant difference was 16.45 mg (between the 0 and 2.0 kG), with medium Aw the difference was 8.09 mg (also between 0 and 2.0 kGy) and with low Aw it was 7.62 mg (between 2.0 and 5.0 kGy). These results demonstrate that the radiolysis has less effect on increasing the amount of phenolics, and less effect on the decreasing of this kind of compound when compared to high Aw.

Analyzing the samples with the same radiation dose, but different Aw, it was possible to verify that in a larger number of doses, the phenolic compounds had not variation among the in the three Aw. At doses of 0, 1.0 and 5.0 kGy the amounts of phenolics were the same in all Aw. Moreover, the values found in medium Aw were more similar to black tea with high Aw than samples with low Aw.

Comparing the radiation doses identified on microbiology to reduce the fungal contamination to not detectable levels, in medium and high Aw (doses of 2.5 and 2.0 respectively) the amount of phenolic compounds was statistically the same to the control samples. The sample with high Aw, despite to need higher radiation dose to decrease the contamination (5.0 kGy) increased the amount of phenolic compounds in this dose.

Regardless the radiation dose and Aw, the values of phenolic compounds quantified are in agreement to Ramalho et al. (2013) who performed the quantification in eight kinds of black tea were

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

| Doses (kGy) | 0.183 | 0.651 | 0.924 |
|---|---|---|---|
| **0** | $84.5 \pm 1.0^{a,x}$ | $84.9 \pm 0.4^{a,c,x}$ | $85.2 \pm 0.1^{a,b,x}$ |
| **1.0** | $96.7 \pm 7.7^{b,c,x}$ | $91.6 \pm 0.3^{b,x}$ | $88.6 \pm 0.9^{b,c,x}$ |
| **1.5** | $94.9 \pm 0.6^{b,c,x}$ | $87.3 \pm 1.0^{a,c,y}$ | $85.6 \pm 1.6^{a,b,y}$ |
| **2.0** | $101.0 \pm 0.2^{c,z}$ | $93.0 \pm 1,5^{b,y}$ | $84.0 \pm 2,9^{a,z}$ |
| **2.5** | $96.8 \pm 3.9^{b,c,z}$ | $87.7 \pm 1.7^{a,c,y}$ | $85.4 \pm 0.3^{a,b,y}$ |
| **5.0** | $93.4 \pm 1.1^{b,x}$ | $92.0 \pm 0.3^{b,x}$ | $91.6 \pm 1.4^{c,x}$ |
| **7.5** | $95.1 \pm 0.1^{b,c,x}$ | $90.6 \pm 0.8^{b,c,y}$ | $88.2 \pm 0.1^{b,c,z}$ |
| **10.0** | $89.9 \pm 0.5^{a,x}$ | $86.4 \pm 2.3^{a,c,y}$ | $87.4 \pm 1.8^{b,y}$ |

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

the results ranged from 50.3 to 274.0 mgGAE/g. 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 found among these authors can be explained due to the fact that several compounds in plants vary according to the age, area/place of cultivations, genetic characteristics of the plant (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 some variation, increasing the amount in some doses and decreasing in other ones, the ORAC assay showed that the radiation up to 10.0 kGy even when combined with different Aw has no interference in antioxidant capacity of black tea (Table 5).

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 the results found in this paper from microbiology, total phenolic compounds, and antioxidant activity assays, the identification and quantification of black tea flavonoids, Gallic acid and caffeine were made only on 5.0 kGy, which was the minimum radiation dose to ensure the microbiological safety in all Aw, and on 10.0 kGy which is the dose usually recommended by several countries and authors for this kind of plant.

The analyses showed that neither the radiolysis nor the direct effect of radiation had influence in the theaflavins, the main flavonoids in black tea, and in Gallic acid (that could be indicate polyphenols break), once no statistical difference was observed at the different radiation doses and Aw used (Table 6). In any samples, the set of catechins and its galates derivate were

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

| Doses (kGy) | 0.183 | 0.651 | 0.924 |
|---|---|---|---|
| **0** | $16.4 \pm 1.4^{ax}$ | $16.5 \pm 1.1^{ax}$ | $16.8 \pm 1.6^{ax}$ |
| **1.0** | $18.4 \pm 1.7^{ax}$ | $16.9 \pm 1.2^{ax}$ | $19.2 \pm 3.9^{ax}$ |
| **1.5** | $18.9 \pm 1.3^{ax}$ | $18.2 \pm 1.4^{ax}$ | $19.8 \pm 4.9^{ax}$ |
| **2.0** | $18.0 \pm 1.9^{ax}$ | $14.4 \pm 0.9^{ax}$ | $17.3 \pm 2.7^{ax}$ |
| **2.5** | $17.4 \pm 2.1^{ax}$ | $17.3 \pm 1.6^{ax}$ | $17.9 \pm 2.4^{ax}$ |
| **5.0** | $17.0 \pm 0.7^{ax}$ | $18.5 \pm 3.7^{ax}$ | $15.7 \pm 0.9^{ax}$ |
| **7.5** | $18.2 \pm 2.3^{ax}$ | $15.7 \pm 4.6^{ax}$ | $15.3 \pm 0.9^{ax}$ |
| **10.0** | $18.1 \pm 1.1^{ax}$ | $16.4 \pm 4.3^{ax}$ | $16.4 \pm 1.3^{ax}$ |

Values represent mean ± standard deviation.

[a] Same superscript letters in the same column mean no statistical difference ($p > 0.05$).

[x] Same superscript letter in the same line mean no statistical difference ($p > 0.05$).

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

| Compounds | Doses (kGy) | Aw | | |
|---|---|---|---|---|
| | | 0.183 | 0.651 | 0.924 |
| **Gallic acid** | **0** | $6.87 \pm 0.17^{a,x}$ | $6.84 \pm 0.14^{a,x}$ | $6.85 \pm 0.13^{a,x}$ |
| | **5.0** | $6.91 \pm 0.20^{a,x}$ | $7.06 \pm 0.10^{b,x}$ | $6.87 \pm 0.10^{a,x}$ |
| | **10.0** | $6.94 \pm 0.21^{a,x}$ | $6.91 \pm 0.07^{a,x}$ | $7.03 \pm 0.28^{a,x}$ |
| **Theaflavins** | **0** | $1.04 \pm 0.12^{a,x}$ | $1.06 \pm 0.06^{a,x}$ | $1.05 \pm 0.06^{a,x}$ |
| | **5.0** | $1.03 \pm 0.12^{a,x}$ | $1.09 \pm 0.06^{a,x}$ | $1.13 \pm 0.07^{a,x}$ |
| | **10.0** | $1.12 \pm 0.14^{a,x}$ | $1.08 \pm 0.06^{a,x}$ | $1.17 \pm 0.08^{a,x}$ |
| **Caffeine** | **0** | $44.6 \pm 0.9^{a,x}$ | $44.4 \pm 0.9^{a,x}$ | $44.7 \pm 1.7^{a,x}$ |
| | **5.0** | $45.4 \pm 0.6^{a,x}$ | $45.2 \pm 0.6^{a,x}$ | $44.9 \pm 0.8^{a,x}$ |
| | **10.0** | $47.2 \pm 0.4^{b,x}$ | $47.3 \pm 0.3^{b,x}$ | $48.3 \pm 3.4^{b,x}$ |

The theaflavins correspond a mix of theaflavin, theaflavin-3 gallate, theaflavin-3′ gallate, and theaflavin-3,3′ digallate compounds.
Values represent mean ± standard deviation.

[a,b] Different superscript letters in the same column mean statistical difference ($p \leq 0.05$).

[x] Same superscript letter in the same line mean no statistical difference ($p > 0.05$).

detected. It can be explained by the process to make the black tea, where the polyphenol oxidase enzyme oxidizes these compounds.

The only compound that presented changes after the radiation processing was the caffeine that had its amount increased when irradiated with 10.0 kGy, in all Aw used. It can be explained by the fact of the caffeine compound is storage within the plant vacuole as a complex with the chlorogenic acids (Baumann and Rohing, 1989; Waldhauser and Baumann, 1996). During the black tea processing, the *C. sinensis* leaves are crushed and ground as goal to break the vacuoles and then release the polyphenol oxidase, which also causes the caffeine release. When the 10.0 kGy radiation dose was applied, the vacuole structures that had not been broken in the black tea manufacturing process, but became fragile, were damage in this dose, causing the increase of caffeine amount that was extract in the infusion processing.

Wang et al. (2010) and Lee et al. (2004) reported granter amounts of theaflavins than this paper; however, the amount observed by Lee and Ong (2000) was similar. These variations of quantity have already been discussed in phenolic compounds item.

## 4. Conclusion

Summarizing, the irradiation with high Aw (0.924) increases the radiation effects on fungal contamination, requiring a lower dose to microbiological control when comparing to a normal Aw and low Aw, without interfering in the flavonoids content and antioxidant activity at doses up to 5.0 kGy.

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