

## EVALUATION OF FUNGI CONTAMINATION AND XANTHINE AND PHENOLIC COMPOUNDS CONTENT IN IRRADIATED MATE (*Ilex paraguariensis*)

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### ABSTRACT

The mate (*Ilex paraguariensis*), a native species from South America, is consumed mainly as beverage, either in a hot (chimarrão) as in cold (tererê) infusion. An important concern is its fungal contamination responsible to affect the physical and nutritional qualities. The radiation processing is effective to reduce the microbiological contamination. The aim of this study was to evaluate the effect of gamma radiation from <sup>60</sup>Co at doses of 0, 3, 5, 7, and 10 kGy in fungal contamination as well as in the nutritional and sensorial characteristics of *Ilex paraguariensis* as chimarrão and tererê beverage. The results show that the dose of 7 kGy decreases the fungal contamination to a safety levels, however decrease the antioxidant activity in chimarrão and promote a change in color characteristic in both beverages. Besides these effects, the dose of 7 kGy is still indicated once the antioxidant activity loss was minimal although the sensorial attributes changed were not percept by consumers.

### 1. INTRODUCTION

The mate (*Ilex paraguariensis*) has been consumed as beverage in a region that comprises Paraguay, Uruguay, North Eastern Argentine and Southern Brazil, as stimulant beverage or for its medicinal properties. The beverage is known as yerba mate, hierba mate, maté, té del Paraguay, erva maté, chimarrão, and tererê [1]. In the last decades, the admiration for mate properties and history has spread to many countries outside of South America including the Middle East, particularly Syria, Lebanon, and Israel [2], Europe, USA, and Japan [3].

The hot infusion is commonly consumed in the cold regions of South America and is prepared from dry ground leaves, been known as chimarrão. However instead of using hot water, mate can be also prepared using cold water (known as tererê) and is consumed mainly in Paraguay and in Brazilian border cities. The leaves are also roasted and used for infusion drinking as it is or made into sweetened drinks [1].

The *I. paraguariensis* chemical composition includes phenols and phenolic acids, amino acids, and other nitrogenated compounds, fatty acids, anthocyanins, flavonoids, terpenic compounds, alcohols, carbohydrates, vitamins, carotenoids, and methylxanthines [4]. The main methylxanthine found in mate is caffeine (0.89–1.73%), followed by theobromine (0.26–0.88%) and small amounts of theophylline. Phenolic compounds constitute another important chemical group in mate, among which chlorogenic acids (CGAs) are the most known. CGAs comprise a family of mono- and di-acyl esters of quinic acid, and the most common acyl residue is the caffeic acid [5].

In the past two decades, there was an increase in the literature studying *I. paraguariensis* once the antioxidants and other compounds shows beneficial effects such as lipoprotein defense against oxidation, antimutagenic, antidiabetics, and antiglycation effects, vasodilating and weight reduction properties [6-8].

Despite a great number of studies on the mycoflora in agricultural products, only a few were concerned with this plant, which are more and more common and plays an important role in the economy in some countries, perhaps the final product presents low water activity. However, Brazilian investigators reported a high contamination of molds, mainly the filamentous fungi for having resistance structures remaining in the product until there are physical conditions for their development [9,10]. Some of those fungi were the *Aspergillus* sp. and *Penicillium* sp. which is well known to produces micotoxins (aflatoxin and ocratoxin, respectively) depending to manipulation and the storage condition.

Ionizing radiation is one of the most effective means to disinfect dry food ingredients. This treatment can inhibit cellular life division, like microorganisms, and promote a molecular structural modification [11,12]. The aims of this study were to evaluate the effects of gamma radiation on fungal decontamination of *Ilex paraguariensis* plant, besides on the xanthine and phenolic compounds content and in the color as physical propriety in *I. paraguariensis* infusion in the form of chimarrão and tererê beverage.

## 2. MATERIAL AND METHODS

### 2.1. Reagents and Standards

Standards (theobromine, caffeine, 5-caffeoylquinic acid [(5-CQA), caffeic acid) were purchased from Sigma-Aldrich (Saint-Louis, USA). Methanol (Sigma-Aldrich, Saint-Louis, USA) was HPLC grade. All other chemicals were analytical grade. Acetic acid was purchased from Sigma-Aldrich (Saint-Louis, USA). Water was purified using a Synergy Milli-Q System (Millipore, USA). Before the use, mobile phase was filtered in a 0.45µm filter (Millipore, USA) and degassed in ultrasonic bath for 30 min.

### 2.2. Samples

The mate herb was donated by Leão Junior S/A (Santa Catarina, Brazil).

### **2.3. Irradiation**

The samples were packed in plastic (polyethylene) bags in the presence of air, sealed and identified with their respective radiation doses. They were irradiated at room temperature (25 °C) in a multi-purpose <sup>60</sup>Co gamma ray facility (IPEN/CNEN-SP) with doses of 0, 3, 5, 7, and 10 kGy with a dose rate of 5.0 kGy/h. Amber 3042 Batch R dosimeters were used to measure the radiation dose.

### **2.4. Water Activity (Aw)**

The Aw were determinate in triplicate using an AQUALAB CX-2 (Decagon Devices, WA, USA) device before and after the irradiation.

### **2.5. Fungal Evaluation**

Fungal analysis was performed adding 10 g of sample in 90 mL of sterile distilled water with agitation for 30 min. A serial dilution from 10<sup>-1</sup> to 10<sup>-6</sup> were prepared and 0.1 mL of each dilution was plated into sterile Petri dish with Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) and Potato Dextrose Agar (PDA) (both from Oxoid, England). Plates were incubated at 25 °C for 5 days [13]. Analysis was performed in triplicate. Fungal counting was performed using a Quebec count equipment and expressed in colonies forming units per gram (CFU/g).

### **2.6. Extraction**

Extracts were prepared using 1.0±0.1 g of herb in 50 mL of distilled water at 85 °C to chimarrão and 10 °C to tererê for 5 min under constant agitation. Each extract was filtered under vacuum into a new recipient and storage at -4 °C until analysis.

### **2.7. HPLC Analysis**

Extracts were diluted at 1:2 and filtered in Millex 0.45 µm filter (Millipore, USA). Analysis were made in a Shimadzu chromatograph with a four pump gradient system (FCV-10AL model) combined with a DAD detector (SPD M10 AVP) and equipped with a C18 reverse column (250 mm x 4,6 mm x 10 µm; Phenomenex, USA). Mobile phase consisted of water and acetic acid (99:1 v/v) (solution "A") and methanol and acetic acid (99:1 v/v) (solution "B") at a flow rate of 0.6 mL/min. An isocratic elution was used (55% of A and 45% of B). An injection volume of 20 µL was used. Detection was performed at 272 nm to xanthine compounds (theobromine and caffeine) and at 323 nm to phenolic compounds (5-caffeoylquinic acid [(5-CQA)] and caffeic acid).

### **2.8. Antioxidant Activity**

For this analysis, samples were prepared under minimal light exposure to prevent degradation. A volume of 1.5 mL of a 20 mg/L DPPH solution (2,2-diphenyl-1-picrylhydrazyl radical) in methanol was added to 0.75 mL of each extract. The absorbance

was determined at 517 nm after 20 min and the scavenging activity was calculated using a standard curve ( $r^2=0.9959$ ) at five different DPPH concentrations. The results were expressed as percentage of the radical reduction (decrease 50% in initial DPPH by the antioxidant) [2].

## **2.9. Color Analysis**

Color measurement was performed in a spectrophotometer (Hunterlab, model ColorQuest XE, USA) with a D65 illumination source and a reading angle of 10°. Experiment was performed in triplicate.

## **2.10. Sensorial Analysis**

The beverages were made using an infusion (50 g herb/L) into hot water (85 °C) to chimarrão and cold water (10 °C) to tererê and stored in separate thermal bottles. The test was done in individual cabins illuminated by fluorescent lamps and the samples were served in transparent plastic cup to 30 untrained volunteers.

The samples were evaluated using a Multiple Difference Test as described by Meilgaard [14]. Briefly, a non-irradiated sample was identified as standard and the irradiated samples plus another non-irradiated sample were codified with a three-digit random number. Panelists were requested to compare the global characteristic (odor, appearance and taste) between the codified samples and the standard sample using a 7 points scale (where 1 = much better than standard and 7 = much worse than standard).

This experiment was approved (protocol number: 221) by the ethic committee of Public Health College of São Paulo University (São Paulo, Brazil).

## **2.11. Statistical Analysis**

The results were expressed as mean±standard deviation. Data was analyzed by ANOVA and Tukey's test using the Prisma 4.0 software (GraphPad) ( $p\leq 0.05$ ).

# **3. RESULTS**

## **3.1. Water Activity**

In the table 1 are shown the water activity values of *I. paraguariensis* herb before and after irradiation.

**Table 1: Water activity values of *I. paraguariensis* before and after the irradiation processing**

Doses (kGy)	Aw
0	0.55 ± 0.02 <sup>a</sup>
3	0.52 ± 0.02 <sup>a</sup>
5	0,54 ± 0,02 <sup>a</sup>
7	0.52 ± 0.02 <sup>a</sup>
10	0.53 ± 0.01 <sup>a</sup>

Same superscripts letters means no statistical difference (p>0.05).

The *I. paraguariensis* herb showed an average Aw value of 0.53. No matter the applied radiation dose, there was no statistical difference (p≤0.05) among the samples. Our results were already expected and are in accordance to several papers which demonstrated no change in Aw after irradiation processing [15-17]. Despite this previous knowledge on Aw variation due to radiation treatment, this measurement was performed because water content is an important tool to correlate to microbiology contamination results [18-22].

### 3.2. Microbiological Analysis

Data on fungi colonies forming units is shown in table 2. According to WHO [23], the acceptable levels of fungi contamination in chimarrão and tererê is 10<sup>5</sup> CFU/g and 10<sup>3</sup> CFU/g, respectively. In this case, in the both medium, the herb can be used to prepare the chimarrão, but not the tererê, although, the values of DRBC medium were higher than the PDA medium. It was observed a decrease in microbial contamination proportional to increasing radiation doses. These results are in line to other authors [12, 24-26] that have already highlighted the inhibitory effect of irradiation in fungi and molds.

**Table 2: Colonies forming units per gram (CFU/g) of fungal contamination in *I. paraguariensis* irradiated and non-irradiated samples using different culture mediums**

Doses (kGy)	Medium	
	DRBC	PDA
0	13 x 10 <sup>3</sup>	8.2 x 10 <sup>3</sup>
3	5.2 x 10 <sup>3</sup>	3.8 x 10 <sup>3</sup>
5	1.6 x 10 <sup>3</sup>	8.0 x 10 <sup>2</sup>
7	3.0 x 10 <sup>1</sup>	1.0 x 10 <sup>1</sup>
10	1.7 x 10 <sup>1</sup>	ND*

\* not detected

Using the PDA medium the dose of 5 kGy was effective to decrease the fungi contamination to safety levels to use in tererê beverage while higher radiation doses was necessary when the herb was seeded in DRBC medium. According to King Jr. [27], DRBC medium is more appropriate than PDA to determinate molds in food because the interactions of dichloran and

rose bengal restrict the diameter of fungal colonies, thus allowing that slow-growing colonies could also develop. In addition, the presence of an antibiotic (chloramphenicol) inhibits bacteria growth. Face to these results, a radiation dose of 7 kGy is recommended to decontaminate *I. paraguariensis* herb, in order to prepare a cold beverage.

### 3.3. HPLC analysis

The chimarrão analysis show that the radiation dose of 10 kGy promoted a decrease of both phenolic compounds content (5-CQA and caffeic acid) ( $p \leq 0.05$ ). Theobromine and caffeine (xanthines) did not show a difference in their content, irrespective the applied radiation dose ( $p > 0.05$ ) (Table 3).

**Table 3: Xanthine and phenolic compounds amount (mg/mL) in non-irradiated and irradiated chimarrão**

Dose (kGy)	Xanthine		Phenolic	
	Theobromine	Caffeine	5-CQA	Caffeic acid
0	$0.038 \pm 0.002^a$	$0.160 \pm 0.010^a$	$0.340 \pm 0.001^a$	$0.013 \pm 0.001^a$
3	$0.037 \pm 0.002^a$	$0.150 \pm 0.010^a$	$0.340 \pm 0.010^a$	$0.012 \pm 0.001^{ab}$
5	$0.038 \pm 0.003^a$	$0.150 \pm 0.007^a$	$0.320 \pm 0.006^{ab}$	$0.011 \pm 0.001^{ab}$
7	$0.038 \pm 0.002^a$	$0.150 \pm 0.011^a$	$0.330 \pm 0.013^{ab}$	$0.012 \pm 0.002^{ab}$
10	$0.032 \pm 0.004^a$	$0.140 \pm 0.009^a$	$0.310 \pm 0.002^b$	$0.010 \pm 0.001^b$

Different superscripts letters in the same column means statistical difference at  $p \leq 0.05$ .

The tererê beverage analysis showed only a statistical decrease for 5-CQA. Xanthine compounds and caffeic acid contents remained similar irrespective the applied radiation dose ( $p > 0.05$ ) (Table 4).

**Table 4: Xanthine and phenolic compounds amount (mg/mL) in non-irradiated and irradiated tererê**

Dose (kGy)	Xanthine		Phenolic	
	Theobromine	Caffeine	5-CQA	Caffeic acid
0	$0.024 \pm 0.003^a$	$0.110 \pm 0.007^a$	$0.250 \pm 0.007^{ab}$	$0.009 \pm 0.001^a$
3	$0.022 \pm 0.001^a$	$0.110 \pm 0.008^a$	$0.270 \pm 0.012^a$	$0.008 \pm 0.001^a$
5	$0.024 \pm 0.003^a$	$0.110 \pm 0.010^a$	$0.260 \pm 0.009^{ab}$	$0.006 \pm 0.001^a$
7	$0.024 \pm 0.001^a$	$0.120 \pm 0.010^a$	$0.250 \pm 0.016^{ab}$	$0.008 \pm 0.001^a$
10	$0.023 \pm 0.001^a$	$0.110 \pm 0.003^a$	$0.240 \pm 0.011^b$	$0.008 \pm 0.001^a$

Different superscripts letters in the same column means statistical difference at  $p \leq 0.05$ .

These findings are in the same range of values to data found in literature. Bastos et al. [28] quantified caffeine and 5-CQA for chimarrão and tererê prepared with non-irradiated mate. They found the same content for these two compounds for tererê beverages, however, they found slightly superior contents for chimarrão beverage. In another study, Bastos et al. [29]

quantified similar amounts of 5-CQA and caffeine in dry mate herb. On the other hand, they found a caffeic acid content inferior than our study. As stated by Cardozo Jr. et al. [5] and Dartora et al. [3], differences in several compounds content could be explained by intrinsic variations in the plant itself, as well as factors such kind of extract, plant age, region of cultivation and genetics features.

Comparing chimarrão to tererê beverage, it is meaningful the high amount of compounds extracted by the former, independently of applied radiation dose. As supported by other authors [30-32], this fact could be easily explained by the higher extraction efficiency of hot water (chimarrão) compared to cold water (tererê beverage).

### 3.4. Antioxidant Analysis

DPPH scavenging activity for chimarrão and tererê prepared with non-irradiated and irradiated herb is shown in table 5. Comparing both beverages, chimarrão showed a higher antioxidant activity than tererê. Irradiation treatment did not have any negative effect on DPPH scavenging activity for tererê ( $p > 0.05$ ), irrespective the applied radiation dose. On the other hand, chimarrão prepared with herb irradiated with radiation doses over 7 kGy showed a decrease in the antioxidant activity ( $p \leq 0.05$ ). It seems that irradiation processing combined to a hot water extraction (chimarrão preparation) decreases the antioxidant activity of *I. paraguariensis*.

**Table 5: DPPH scavenging activity (IC50) ( $\mu\text{g/mL}$ ) of non-irradiated and irradiated chimarrão and tererê extracts**

Doses (kGy)	Chimarrão	Tererê
0	27.67 $\pm$ 0.11 <sup>a</sup>	36.96 $\pm$ 0.92 <sup>a</sup>
3	27.90 $\pm$ 0.21 <sup>a</sup>	35.78 $\pm$ 0.29 <sup>a</sup>
5	29.27 $\pm$ 0.58 <sup>ab</sup>	36.54 $\pm$ 0.07 <sup>a</sup>
7	30.67 $\pm$ 0.97 <sup>b</sup>	35.62 $\pm$ 0.26 <sup>a</sup>
10	31.22 $\pm$ 0.54 <sup>b</sup>	36.69 $\pm$ 0.17 <sup>a</sup>

Different superscripts letters in the same column means statistical difference at  $p \leq 0.05$ .

In a previous work, we observed that radiation processing enhanced the total phenolic compounds (5-CQA equivalent) in tererê samples at radiation levels of 3 kGy and 7 kGy [33]. This increase in total phenolic compounds could be related to a release of compound from glycosidic components by the radiation, “working” as an enzyme.

Mishra et al. [16] analyzed the effect of radiation processing at doses up to 10 kGy in *Camellia sinensis* leaves and showed similar results, suggesting that irradiation treatment has a variable effect on phenolic compounds depending on radiation doses applied. Other studies evaluated phenolic compounds in irradiated plants, presented no significant loss of these compounds [34,35].

### 3.5. Color Analysis

Color analysis showed that in chimarrão, the irradiation at 10 kGy significantly change this characteristic making the infusion more light (“L” value) than control sample and the 5 kGy; the dose of 7 kGy become more reddish (“a” value) than other doses and together with 10 kGy made the beverage more yellowish (“b” value) (Table 6). In tererê the dose of 10 kGy has opposite effect in brightness value making the beverage darker than control sample and no difference was found on the others color parameters (Table 7).

**Table 6: Color measurement values (L, a, b) of non-irradiated and irradiated chimarrão**

Doses (kGy)	Chimarrão		
	L	a	b
0	47.51 ± 0.89 <sup>a</sup>	2.90 ± 0.27 <sup>a</sup>	23.42 ± 1.94 <sup>ab</sup>
3	49.24 ± 1.12 <sup>ab</sup>	2.98 ± 0.36 <sup>a</sup>	21.61 ± 2.02 <sup>a</sup>
5	46.49 ± 1.52 <sup>a</sup>	3.15 ± 0.29 <sup>ab</sup>	22.00 ± 1.68 <sup>a</sup>
7	49.88 ± 0.78 <sup>ab</sup>	3.79 ± 0.35 <sup>b</sup>	24.97 ± 1.73 <sup>b</sup>
10	52.33 ± 1.94 <sup>b</sup>	3.13 ± 0.23 <sup>ab</sup>	25.84 ± 1.95 <sup>b</sup>

Different superscripts letters in the same column means statistical difference at  $p \leq 0.05$ .

**Table 7: Color measurement values (L, a, b) of non-irradiated and irradiated tererê**

Doses (kGy)	Tererê		
	L	a	b
0	63.77 ± 1.80 <sup>a</sup>	-0.77 ± 0.33 <sup>a</sup>	22.74 ± 0.95 <sup>a</sup>
3	61.64 ± 1.74 <sup>ab</sup>	-0.23 ± 0.25 <sup>a</sup>	21.61 ± 0.69 <sup>a</sup>
5	60.66 ± 2.11 <sup>ab</sup>	-0.47 ± 0.37 <sup>a</sup>	21.50 ± 0.98 <sup>a</sup>
7	61.33 ± 0.94 <sup>ab</sup>	-0.38 ± 0.41 <sup>a</sup>	21.25 ± 1.13 <sup>a</sup>
10	57.14 ± 2.14 <sup>b</sup>	-0.27 ± 0.22 <sup>a</sup>	21.40 ± 0.84 <sup>a</sup>

Different superscripts letters in the same column means statistical difference at  $p \leq 0.05$ .

Jo et al. [36] demonstrated that green tea leaves (*C. sinensis*) extracts irradiated with doses up to 20 kGy had the color enhanced proportionally to increasing doses, although no difference between irradiated and non-irradiated when they performed the antioxidant activity analysis.

### 3.6. Sensorial analysis

As previously discussed, although irradiation treatment altered somehow chemical composition and physical status of chimarrão and tererê, no negative effect was found on sensorial aspects of both beverages at dose up to 10 kGy. As summarized in table 8, consumers perception evaluated by sensorial analysis enlightened that irradiation did not change negatively sensorial aspects of both beverages ( $p > 0.05$ ).

**Table 8: Global score averages (odor, appearance and taste) of chimarrão and tererê prepared with non-irradiated and irradiated herb**

Doses (kGy)	Chimarrão	Tererê
0	3.80 <sup>a</sup>	4.14 <sup>a</sup>
3	3.90 <sup>a</sup>	3.76 <sup>a</sup>
5	4.16 <sup>a</sup>	4.17 <sup>a</sup>
7	4.40 <sup>a</sup>	3.97 <sup>a</sup>
10	3.80 <sup>a</sup>	4.14 <sup>a</sup>

Same superscripts letters means no statistical difference (p>0.05).

Despite the statistical difference on color parameters related before, panelists were not able to distinguish any difference among non-irradiated and irradiated samples, for both kinds of infusion.

Our results are in line to Misha et al. [16] whom demonstrated that a radiation dose of 5 kGy was effective to decontaminate green tea (*C. sinensis*) with no changes on antioxidant activity and sensorial characteristics at doses up 10 kGy. Later, Fanaro et al. [37,38] also observed no negative effect of gamma radiation (0, 5, 10, 15, and 20 kGy) on sensorial features of green tea and oolong tea (partial fermented *C. sinensis*). They also highlighted that volatile organic compounds profile of green tea remained unaltered over this radiation dose range. On the other hand, a statistical difference in volatiles profile was found for 5 kGy and 15 kGy irradiated samples.

#### 4. CONCLUSIONS

Despite the radiation dose of 7 kGy decrease the antioxidant activity in chimarrão and promote changes in color characteristics in both beverages used, this dose is recommended once the nutritional losses is minimal compared to the microbiology safety gain, beyond that consumers can not perceive the physical changes promoted by this dose.

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