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Effect of gamma radiation on antioxidant capacity of green tea, yerba mate, and chamomile tea as evaluated by different methods

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HIGHLIGHTS

- Gamma-radiation generated free radicals on three teas matrices.
- Irradiation preserved antioxidant activity although some activities were diminished.
- The evaluation of antioxidant capacity depends on the methodology chosen.

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ABSTRACT

Tea is a traditional plant extract with important cultural ties. It is the most widely consumed beverage in the world. Tea consumption has some health benefits including antioxidant stimulus. Gamma radiation is currently used to control of postharvest pathogens on tea herb. However, free radicals can be generated, which consumes antioxidant molecules. A positive relation was found between radiation doses used and free radicals generation in green tea (*Camellia sinensis*), yerba mate (*Ilex paraguariensis*), and chamomile tea (*Matricaria recutita*). Total antioxidant capacity (TAC) of aqueous and methanol extracts of these herbs was determined by various methods to compare the effect of irradiation of herb on antioxidant capacity of the extracts. TAC was evaluated by measuring: total phenols (decreased with irradiation in mate and green teas), total flavonoids (stable in aqueous extracts and decreased with irradiation in methanol extract of mate and chamomile), Trolox equivalent or ABTS (unchanged under irradiation), DPPH* scavenging capacity (stable on aqueous extract but diminished in methanol extract after irradiation), β carotene/acid linoleic ability (stable with the exception of chamomile tea that increased after irradiation) and, capacity to chelate ferrous ions (unchanged with irradiation). In conclusion, gamma irradiation reduced the capacity of some antioxidants but preserved the capacity of others. This study showed that one isolated test does not suffice to perform this evaluation reliably, which is a reflection of the diversity and complexity of the effects of irradiation on antioxidant molecules present in different samples.

1. Introduction

More tea is consumed than any other beverage than water. Tea is mainly consumed for cultural reasons and because of its health benefits. People around the world consume tea daily throughout their lives. There are many different ways to prepare tea, but the leaves, flowers, or roots of the plants normally undergo an infusion process using hot water. The plant most commonly used to prepare tea is *Camellia sinensis*. *Camellia sinensis* are used in different preparation methods to produce white, black, green, or oolong tea (Graham, 1992). In South America, the best-known and most popular variety is *Ilex paraguariensis*, which is used to make yerba mate. It is used to prepare infusions or decoctions. Chamomile

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(*Matricaria recutita*) has been a common medicinal plant for centuries (Srivastava and Gupta, 2007). Clinical trials have verified its medicinal properties. Tea has been considered to be healthy since ancient times. Today, many studies have shown that drinking tea is a healthy habit that can help to prevent some lifestyle-related diseases such as cancer, obesity, cardiovascular disease, inflammation, diabetes. The active substances present in tea are polyphenols. Polyphenols have many beneficial properties. They have antioxidant, anti-mutagen, anti-estrogenic, anti-carcinogenic, and anti-inflammatory effects that have the potential to prevent disease (Ferguson, 2001). Polyphenols are the general denomination for a large group of molecules. The type of polyphenol depends on the herb used. Green tea contains epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC), and flavonoids including quercetin, kaempferol, and myricitin (Graham, 1992). Yerba mate contains different types of polyphenol, xanthine, and saponin, as well as caffeoyil derivatives (Heck and De Mejia, 2007). Chamomile tea contains several phenol compounds, such as apigenin, quercetin, patuletin, and luteolin, and their glucosides (McKay and Blumberg, 2006).

Food irradiation is known to be a safe method of treating food products using ionizing radiation. Gamma rays is employed to prolong the shelf-life of food. It lead to the inactivation of food-borne pathogens, which allows products to pass through quarantine barriers in international trade because the product is made more hygienic and contamination of local species is avoided (Farkas, 2006). However, irradiation procedures can generate free radicals (Dainton, 1948). All cells contain antioxidant molecules that block or slow the reaction of free radicals with the main components of cells (lipids, proteins, nucleic acids, and carbohydrates) by consuming free radicals. Antioxidant molecules become depleted in the process (Halliwell, 1994). Studies of the correlation between irradiation disinfection, inhibition of spores, and extension of shelf-life are well known and have been discussed in several reviews (Farkas, 1998 and WHO, 1981). In general, the antioxidant capacity of dry tea leaves is proportionally lower after irradiation. The purpose of this study was to determine how gamma irradiation affects the total antioxidant capacity present in dry leaves or flowers of the three types of tea cited above by using some typical methodologies.

2. Materials and methods

2.1. Chemical products

Ferrous chloride, ferric chloride, gallic acid, aluminum trichloride, quercetin, trolox, chloroform, β-carotene, linoleic acid, tween 80, ferrozine, Folin–Ciocalteu's reagent (FCR), methanol, trichloroacetic acid (TCA), 1.1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), butylated hydroxytoluene (BHT), and butylated hydroxyanisol (BHA) were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents are of analytical grade.

2.2. Tea herb samples

Sachets of green tea, yerba mate, and chamomile were purchased at local markets. The sachets were opened by hand; the contents were mixed and then sieved (42 and 100 mesh). The prepared tea then directly underwent gamma irradiation.

2.3. Gamma irradiation treatment

The sieved samples were irradiated in a circular Gamma Irradiator (GB-127 IR-214, MDS Nordion, Canada) at CDTN (a unit of the Brazilian Authority of Nuclear Energy) with a ^{60}Cobalt source at a dose rate of 15 Gy/h. Samples (10 g) of powder were placed in polyethylene flasks and irradiated at standard temperature and pressure.

2.4. EPR measurements

20 ± 2 mg of prepared tea herb was placed in quartz tubes with an inner diameter of 2 mm. EPR measurements were performed on a Magnettech spectrometer with a rectangular cavity (located in the Physics Department at UFMG, Brazil). The microwave frequency was $\nu \approx 9.4$ GHz, magnetic field modulation was 100 kHz, modulation amplitude was 2 G, and power was 10 mW at room temperature. EPR measurements of control samples that had not been irradiated and irradiated powder samples were measured several times beginning about 2 h after irradiation until 14 days after irradiation to evaluate the stability of EPR signals over time.

2.5. Methanol extract preparation

1 g of the prepared tea herb was incubated with 40 mL of methanol 50%. The solution was homogenized and left at rest at room temperature for 60 min. The samples were then centrifuged ($15\ \text{min} \times 2000g$) and the supernatant was reserved. 40 mL of acetone 70% were added to the pellet. After 60 min the supernatant was pooled and water was used to increase the volume to 100 mL. This preparation was designated methanol extract. It was used in all TAC tests.

2.6. Aqueous extract preparation

100 mL of ultra-pure water at 98 °C was added to 1 g of prepared tea herb. After it had been gently homogenized, the solution was left to settle for 60 min at room temperature. It was then centrifuged ($15\ \text{min} \times 2000g$) and the supernatant was reserved for TAC measurement. This preparation was designated aqueous extract.

2.7. Total antioxidant capacity (TAC)

Different methods were used to analyze TAC.

2.7.1. Test for total phenols content

Total phenols content was measured using the Folin-Ciocalteau colorimetric method (Chandler and Dodds, 1983). The extract (0.5 mL) was mixed with 45 mL of distilled water and 1 mL of Folin-Ciocalteau reagent. Then 3 mL of 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking at room temperature. The absorbance of the mixture at 760 nm was recorded by a spectrophotometer (model UV 1600 PC; Shimadzu, Japan). Total phenols content was expressed as gallic acid equivalent (GAE) obtained from the standard gallic acid curve (2, 4, 6, and 8 μg/mL). Each extract sample was measured in triplicate.

2.7.2. Test for total flavonoids

This test used the method described by Arvouet-Grand et al. (1994). To summarize, 2% aluminum trichloride ($AlCl_3$) dissolved in 1 mL of methanol (100%) was mixed with 1 mL of the methanol extracts and aqueous extract. Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 1 mL extract solution with 1 mL methanol that did not contain $AlCl_3$. The concentration was calculated using the equation obtained