Radiation Physics and Chemistry 81 (2012) 1152–1156

Contents lists available at SciVerse ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Evaluation of γ-radiation on oolong tea odor volatiles

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ARTICLE INFO

Article history:
Received 17 June 2011
Accepted 26 November 2011
Available online 6 December 2011

Keywords:
Oolong tea
Camellia sinensis
Volatile organic compounds
Food irradiation

ABSTRACT

The aim of this study was to evaluate the gamma radiation effects on odor volatiles in oolong tea at doses of 0, 5, 10, 15 and 20 kGy. The volatile organic compounds were extracted by hydrodistillation and analyzed by GC/MS. The irradiation has a large influence on oolong tea odor profile, once it was identified 40% of new compounds after this process, the 5 kGy and 20 kGy were the doses that degraded more volatiles found naturally in this kind of tea and the dose of 10 kGy was the dose that formed more new compounds. Statistical difference was found between the 5 kGy and 15 kGy volatile profiles, however the sensorial analysis showed that the irradiation at dose up 20 kGy did not interfere on consumer perception.

1. Introduction

Tea is a widely popular beverage consumed in the world for over several thousand years after water (Dou et al., 2007). Reasons for its great popularity range from cultural traditions to supposed health benefits. Results from epidemiological studies as well as laboratory experiments suggest that consumption of tea confers protection against the development of chronic diseases, such as cardiovascular and cancer (Santana-Rios et al., 2001).

Tea is processed from tender shoots of *Camellia sinensis* (L.) O. Kuntze and is mainly classified into green tea (unfermented), oolong tea (partially fermented) and black tea (fully fermented) according to the degree of fermentation during their preparations, where the term "fermentation" refers to natural browning reactions induced by oxidative enzymes in the cells of tea leaves. Oolong tea, possessing a taste and color somewhere between green and black teas, is manufactured predominantly in China and Taiwan (Lee et al., 2008).

Among the benefits associated to oolong tea consumption are the increasing of energy expenditure (acting on weight loss), the prevention of cardiovascular disease (Yamamoto et al., 2000), reduce the LDL oxidation, total cholesterol and triglyceride and may be beneficial in the prevention of coronary heart disease (Hosoda et al., 2000), anti-cancer, anti-oxidant, anti-inflammatory and anti-bacterial activity (Chen et al., 2010) and suppressing the development of hypertension in rats (Tanida et al., 2008).

People enjoy tea for its taste and flavor. Therefore, most studies conducted on tea showed taste and flavor investigations. In the 1970s and 1980s, many researchers analyzed the flavor constituents of various tea extracts (Yanagimoto et al., 2003).

Flavor comprises, principally, taste and aroma. Non-volatile components are generally responsible for the taste, while volatile components give the aroma. In tea, volatile organic compounds (VOC) are present in minimum quantities (0.01% of the total dry weight), but have a high impact on the flavor due to their low threshold value and resulting high odor units. VOC of tea are classified into two groups: group I, consisting mainly of non-terpenoids and group II, terpenoids, which impart sweet flowery aroma to tea, viz, monoterpene alcohols such as linalool and geraniol. The presence of group II compounds is highly desirable (Ravichandran and Parthiban, 1998; Rawat et al., 2007).

The past decade had a significant increase in the use of herbal medicine. Despite a great deal of studies on the mycoflora in agricultural products, only a few were concerned with spices and medicinal herbs, which are more and more common in our daily diet and play an important role in the economy in some countries (Romagnoli et al., 2007), like oolong tea. Nevertheless, some investigators reported the presence of molds in medicinal plants, herbs and natural drugs that are widely consumed not only as home medicine, but as raw materials for the pharmaceutical industries as well (Aquino et al., 2007; Roy and Chourasia, 1989).

In some kinds of medicinal plants analyzed, the percentage of contamination in packed samples was higher than in those non-packed; this may be caused by other factors, such as, humidity

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doi:10.1016/j.radphyschem.2011.11.061

inside the pack or unsuitable methods of keeping and storing the pack (Abou-Arab et al., 1999).

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 (Farkas, 2006; Villavicencio et al., 2007). The aim of this study was to evaluate the effects of gamma radiation on odor compounds in oolong tea.

2. Material and methods

2.1. Samples

The oolong tea was purchased from a market in a traditional oriental neighborhood of São Paulo, Brazil.

2.2. 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 in a ^{60}Co source Gammacell 220 (Nordion Ltd., Canada) with a dose rate of 2.39 kGy/h, at doses of 0, 5, 10, 15 and 20 kGy. Harwell Amber 3042 dosimeters were used to measure the radiation dose. To VOC analysis, the samples were processed in triplicate.

2.3. Extraction of VOC by hydrodistillation

100 g of oolong tea was placed in a round-bottom flask with 700 mL of boiling distilled water. The contents were distilled for 60 min to collect the volatile oil. The essential oil was separated from the aqueous phase by dichloromethane (10 mL) for 60 min, dried over pure nitrogen flow, re-suspended with 1 mL of dichloromethane and stored in a freezer (-4 °C) (Fanaro et al., 2011).

2.4. Gas chromatography–mass spectrometry (GC–MS) analysis

The concentrated extracts were analyzed in a HP 5973MSD detector coupled to a HP 6980 GC. An HP-5MS column (30 m/ 0.25 mm id, film thickness 0.25 μm) was used with helium as a carrier gas. The injector temperature was 200 °C in splitless mode.

The GC oven temperature was programmed to hold at 50 °C for 0.5 min and then to increase to 250 °C at 5 °C/min, finally holding at 250 °C for 0.5 min. Column flow rate was 1 mL/min. Ion source temperature was 230 °C and the interface temperature was set at 200 °C. The MS was scanned at 70 eV over 30–550 a.m.u. Sample injection volume was 1.0 μL.

The volatile identification were performed by the comparison between the mass spectrum of the compounds with the NIST 98 library database and their respective retention index with values found in the literature. The retention index was calculated as described by Ettre (2003) with an alkane solution containing C_8–C_{40} (Sigma-Aldrich, St. Lois, USA) as standard. Once the compounds were identified, each VOC had its odor characteristic description found in the scientific literature.

2.5. Sensorial analysis

The samples were prepared by infusion into a boiling water contend 10 g/L of tea for 5 min, filtered using paper filters and stored in thermal bottles of 1 L.

The test was performed in individual cabins illuminated by fluorescent lamps. Samples were served in plastic cup, codified with a three-digit random number, with a glass of water to 25 untrained volunteer panelists. The samples were evaluated using a preference test based on a nine-point hedonic scale (9 = like extremely and 1 = dislike extremely), and the global characteristics (odor, appearance and taste) were measured.

This experimental was approved (protocol number: 1985) by the ethic committee of Public Health College of São Paulo University (São Paulo, Brazil).

Although several studies have reported that the boiling water in the infusion preparation decreases the antioxidant activity, the boiling water was used due to the fact that it is described in the preparation instruction in several teas packaging, being so, how consumers usually prepare their beverage.

2.6. Statistical analysis

The principal component analysis was performed to evaluate the volatile compounds profiles and the ANOVA to sensorial analysis. In both case, was use the value of $p \leq 0.05$.

3. Results and discussion

A total of 123 volatiles were identified in oolong tea and the half of this compounds (49.6%, $n=61$) identified was formed by irradiation. However, only 40 compounds (32.5%) have some kind of odor (Table 1). The odor description of all compounds found in this paper (on irradiated and non irradiated samples) is described in literature.

The majority compounds identified with odor (60.0%, $n=24$) were the compounds found naturally on oolong tea, i.e. in non irradiated samples, however three of these volatiles (4-acetyltoluene, geranial and δ-dodecalactone) were degraded by 5 kGy, one ((E,E)-2,4-heptadienal) by 15 kGy and the β-ciclocitral; 1,1, 6-trimethyl-1,2-dihydronaphthalene and eugenol by 20 kGy, totaling seven compounds that were degraded by gamma radiation (Fig. 1).

Despite the dose of 10 kGy did not degrade any compounds found naturally in oolong tea, it was the radiation dose that most formed new volatiles ($n=6$) with odors; however, only the (E,E)-2,4-decadienal was resistant to higher doses. The doses of 15 kGy and 20 kGy formed four compounds each and among the volatiles formed by 15 kGy only the safranal was resistant to the highest dose used in this work. The 5 kGy was the dose that formed less compounds, only two, and, like happened with the other doses, only one volatile (2-acetylpyrrole) was resistant when higher dose was applied.

The principal component analysis shows that the doses of 15 kGy and 20 kGy showed a profile more related with the control sample than the other doses applied and the dose of 20 kGy was the dose that had more similarities with all doses. A statistical difference ($p \leq 0.05$) can be considered between the 5 kGy and 15 kGy doses, due the distance presented between these two doses (Fig. 2).

Fanaro et al. (2011), working to green tea, shows a different behavior of volatiles compounds, although it was the same plant used (*C. sinensis*) and the same radiation doses. In their work, the major compounds formed were resistant when applied higher doses and no statistical difference was detected among all doses, demonstrating that the enzymatic process, even partially, has a impact on volatiles compounds behavior when irradiated.

Most of the compounds ($n=19$) found naturally in oolong tea in this work, has some kind of pleasant odor. Among the pleasant odors are the flavors of almond/caramel (5-methylfurfural and 4-acetyltoluene), sweet (caproic acid and octanoic acid), potato (2,5-dimethyl-3-ethylpyrazine), honey (phenethyl alcohol), peppermint/mint (methyl salicylate and β-ciclocitral), lemon (geranial), green (nonanoic acid), licorice (1,1,6-trimethyl-1,2-dihydronaphthalene), clove

Table 1
Volatile organic compounds with odors identified in oolong tea at different radiation doses.

#CAS	RT (min)	RI	Compound	Doses (kGy)					Odor	Reference[a]
				0	5	10	15	20		
100-52-7	6.50	975	Benzaldehyde			x			Almond, Caramel	Schieberle and Grosch, 1987
620-02-0	6.56	977	5-Methylfurfural	x	x	x	x	x	Almond, Caramel	Schieberle and Grosch, 1987
142-62-1	7.40	999	Caproic acid	x	x	x	x	x	Sweet	Adedeji et al., 1991
4313-03-5	7.75	1011	(E,E)-2,4-Heptadienal	x	x	x			Fried	Ullrich and Grosch, 1988
1072-83-9	9.17	1061	2-Acetylpyrrole		x	x	x	x	Nut, Walnut, Bread	Yong et al., 1989
13360-65-1	9.69	1079	2,5-Dimethyl-3-ethylpyrazine	x	x	x	x	x	Potato	Schieberle and Grosch, 1987
90-05-1	9.95	1088	Guaiacol					x	Smoke, Medicine	Ong and Acree, 1999
78-70-6	10.29	1100	Linalool				x		Flower, Lavender	Schieberle and Grosch, 1987
60-12-8	10.63	1112	Phenethyl alcohol	x	x	x	x	x	Honey, Spice, Rose	Blank et al., 1989
123-07-9	12.21	1168	4-Ethylphenol					x	Spice	Aznar et al., 2001
122-00-9	12.22	1168	4-Acetyltoluene	x					Bitter almond	Schieberle and Grosch, 1987
95-93-2	12.32	1172	Durol			x			Rancid	Luning et al., 1994
124-07-2	12.68	1184	Octanoic acid	x	x	x	x	x	Sweat, Cheese	Adedeji et al., 1991
119-36-8	12.86	1191	Methyl salicylate	x	x	x	x	x	Peppermint	Berger et al., 1989
116-26-7	13.03	1196	Safranal				x	x	Herb, Sweet	Jorgensen et al., 2000
5910-87-2	13.44	1212	2,4-Nonadienal			x			Fat, Wax	Ullrich and Grosch, 1987
432-25-7	13.58	1217	β-Ciclocitral	x	x	x	x		Mint	Berger et al., 1989
502-99-8	13.85	1227	α-Ocimene				x		Fruit	Jordan et al., 2003
122-03-2	14.12	1237	4-Isopropylbenzaldehyde					x	Acid	Chung et al., 1993
3913-81-3	14.74	1261	(E)-2-Decenal				x		Tallow	Gasser and Grosch, 1990
141-27-5	14.96	1269	Geranial	x					Lemon, Mint	Schieberle and Grosch, 1987
25773-40-4	15.07	1273	Isopropylmethoxypyrazine					x	Pea, Earth	Blank and Grosch, 1991
112-05-0	15.33	1283	Nonanoic acid	x	x	x	x	x	Green	Jirovetz et al., 2002
120-72-9	15.60	1293	Indole	x	x	x	x	x	Mothball, Burnt	Gasser and Grosch, 1990
25152-84-5	16.19	1315	(E,E)-2,4-Decadienal			x	x	x	Fried, Fat	Ullrich and Grosch, 1987
30364-38-6	17.10	1349	1,1,6-Trimethyl-1,2-dihydronaphthalene	x	x	x	x		Licorice	Jorgensen et al., 2000
97-53-0	17.27	1356	Eugenol	x	x	x	x		Clove, Honey	Blank et al., 1989
97-54-1	17.27	1356	Isoeugenol	x	x	x	x	x	Flower	Nishimura, 1995
334-48-5	17.73	1373	Capric acid		x				Rancid, Fat	Miranda-Lopez et al., 1992
83-34-1	17.98	1382	3-Methylindole	x	x	x	x	x	Mothball	Widder et al., 1991
529-01-1	18.85	1417	Isopiperitenone			x			Mint	Jirovetz et al., 2002
3796-70-1	19.70	1453	Geranyl acetone	x	x	x	x	x	Magnolia, Green	Mau et al., 2003
14901-07-6	19.78	1456	β-Ionone	x	x	x	x	x	Violet, Raspberry	Berger et al., 1989
713-95-1	20.70	1495	δ-Dodecalactone	x					Fruit, Sweet	Moio et al., 1994
7212-44-4	22.37	1565	Nerolidol	x	x	x	x	x	Wood, Flower	Chung et al., 1993
120-51-4	26.71	1763	Benzyl benzoate			x			Balsamic, Herb	Adedeji et al., 1991
502-69-2	28.40	1954	Phitone	x	x	x	x	x	Fat	Mau et al., 2003
762-29-8	29.85	1881	Farnesylacetone	x	x	x	x	x	Flower, Ether	Mau et al., 2003
150-86-7	33.48	1696	Phytol	x	x	x	x	x	Flower	Chung et al., 1993
112-80-1	33.93	1674	Oleic acid	x					Fat	Berger et al., 1989

[a] The references in this table correspond to the papers that describe the compounds odors.

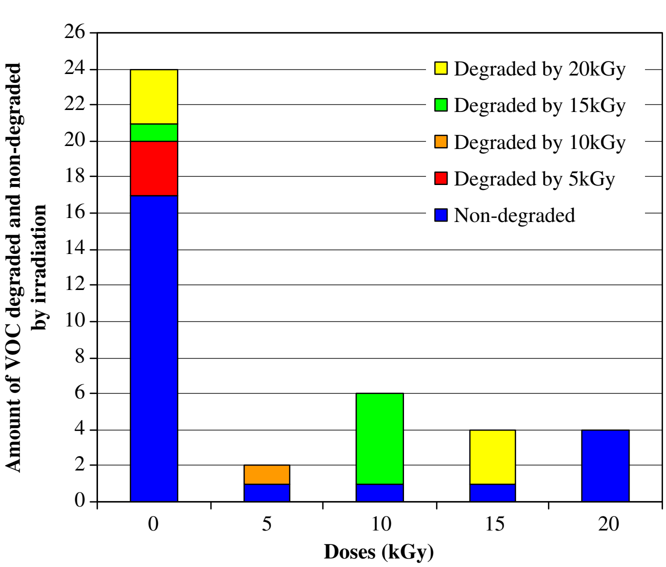

Fig. 1. Amount of VOC, with odor, degraded and non-degraded in oolong tea after the irradiation with different doses.

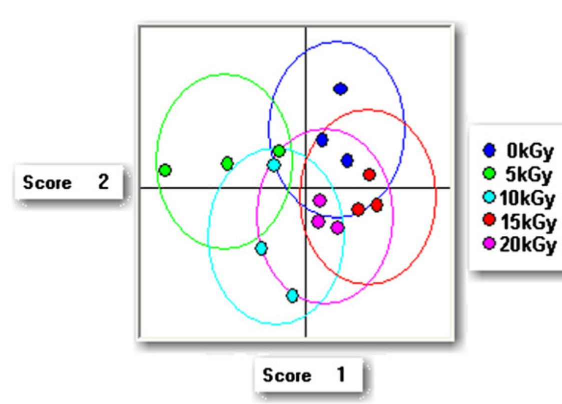

Fig. 2. Principal components analysis from oolong tea irradiated with different doses.

(eugenol), flower (isoeugenol, geranyl acetone, β-ionone, farnesylacetone and phytol), fruit (δ-dodecalactone), and wood (nerolidol) (Fig. 3, Table 1).

The half compounds formed by 5 kGy, 10 kGy and 20 kGy, has some pleasant odor as nut/bread (2-acetylpyrrole) formed by

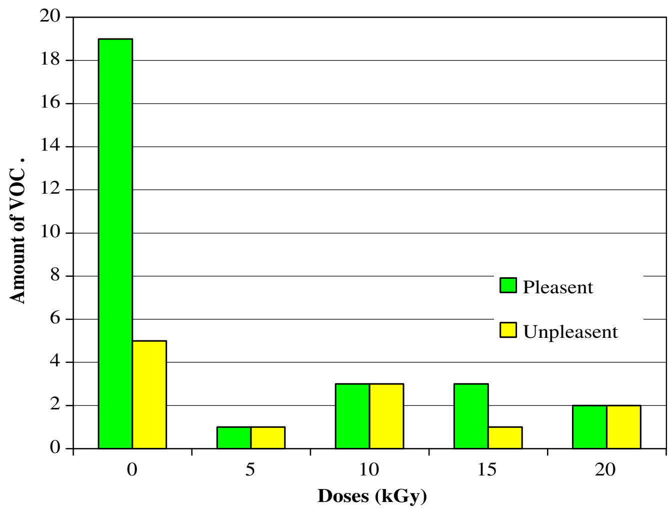

Fig. 3. Amount of volatiles organic compounds that have pleasant and unpleasant odors found in irradiated and non-irradiated oolong tea.

Table 2
Global (odor, appearance and taste) score averages of oolong tea irradiated with different radiation doses.

Doses (kGy)	0	5	10	15	20
Score average	3.7 ± 1.8[a]	4.4 ± 2.1[a]	4.5 ± 2.0[a]	4.5 ± 2.0[a]	4.3 ± 2.3[a]

[a] Same letter means no statistical difference at $p \leq 0.05$.

5 kGy; almond/caramel (benzaldehyde), mint (isopiperitenone) and balsamic/herb (benzyl benzoate) formed by 10 kGy and spice (4-ethylphenol) and pea (isopropylmethoxypyrazine) that was formed by 20 kGy. The dose of 15 kGy formed more pleasant than unpleasant odors. Among the pleasant odors are the aromas of flower (linalool), herb (safranal) and fruit (α-ocimene).

Among the unpleasant odors formed by radiation, were found aromas of rancid/fat (capric acid) formed by 5 kGy; rancid (durol), wax (2,4-nonadienal) and fried ((E,E)-2,4-decadienal) formed by 10 kGy, tallow ((E)-2-decenal) formed by 15 kGy and smoke (guaiacol) and acid (4-isopropylbenzaldehyde) formed by 20 kGy.

Even the radiation doses have formed unpleasant odors, it does not mean that these compounds have a negative impact on the tea flavor. Compounds with unpleasant odors were also found in the control sample as the (E,E)-2,4-heptadienal (fried odor), indole and 3-methylindole (mothball odor) and phitone and oleic acid (fat odor). The same theory is true for compounds with pleasant odors formed after the irradiation, meaning that they do not make a better tea aroma. For this, a sensory analysis (Table 2) was performed to evaluate if the compounds formed by radiation, pleasant or unpleasant, has a directly interference on consumers perception.

Despite the statistical difference ($p \leq 0.05$) found on volatile profile, the consumers were not able to distinguish any difference among the samples tested. The sensorial analysis results showed even a slight preference to the samples irradiated, noting that the unpleased compounds formed by the irradiation did not interfere on consumer perception.

Furgeri et al. (2007) studying the sensorial analysis in maté (*Ilexis paraguariensis*), irradiated at doses up to 10 kGy, demonstrated that there was no difference between the irradiated and non-irradiated samples. Salum et al. (2007) showed in their sensorial analysis that the participants had difficulties to define among the differences in odor between the control and irradiated cinnamon and nut meg.

4. Conclusion

The gamma radiation showed has a direct influence on volatile compounds profile, increasing VOC quantity, degrading few compounds and producing even pleased as unpleased odors that are not found naturally in oolong tea. Statistical difference was found between the 5 kGy and 15 kGy volatile profiles ($p \leq 0.05$), however the sensorial analysis showed that the irradiation at doses up 20 kGy has no influence on consumer perception in this kind of tea.

Acknowledgment

We are thankful for IPEN, CNEN, CAPES and CNPq for financial support.

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