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Relationship between photon emission and chemopreventive potential of tea

Yumiko Yoshiki [a,*], Naohiko Onda [a], Kazuyoshi Okubo [b]

[a] *Laboratory of Biostructural Chemistry, Graduate School of Life Science, Tohoku University, 1-1 Tsutsumidori Amamiyamachi, Aoba-ku, Sendai, Miyagi 981-8555, Japan*
[b] *Department of Complementary and Alternative Medicine, Graduate School of Medical Science, Kanazawa University, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8640, Japan*

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Abstract

Photon emission from tea (610–670 nm) in the H_2O_2/$KHCO_3$/MeCHO system and the utility of the photon detection system in tea evaluation were investigated. Photon intensity of tea depended on manufacturing method as follows: green tea ($1202 \pm$ SD 173.51 cd/m^2) > oolong tea ($834 \pm$ SD 237.44 cd/m^2) > black tea ($222 \pm$ SD 87.65 cd/m^2). Photon intensity corresponded with polyphenol content ($r^2 = 0.8810$) rather than catechin content ($r^2 = 0.7759$) and showed a high correlation with chemopreventive activities against H_2O_2 ($r^2 = 0.7516$), O_2^- ($r^2 = 0.7998$) and DPPH radical ($r^2 = 0.7516$). Photon intensity from green tea leaves at each manufacturing process step gave the same decreasing curve as DPPH radical scavenging activity. Chemiluminescence of tea, which gives information on the polyphenol content and chemopreventive potential, is a useful technique for tea evaluation.

1. Introduction

Chemiluminescence in analytical chemistry has received much attention as an analytical technique for the detection of deterioration of dietary lipids (Loeliger & Saucy, 1984; Yamamoto, Brodsky, Baker, & Ames, 1987; Benov & Ribarov, 1990), the determination of Ni(II), Cr(III) and Mo(VI) metals in water (Lu, Lu, & Zhao, 1991; Escobar, Lin, Guiraum, & de la Rosa, 1993; Navas & Jimenez, 1996), and the detection of anabolic steroids in meat (Jansen, Bergman, Van den Berg, & Zomer, 1989). A superior sensitivity analysis of chemiluminescence, as applied to food, has been extensively reviewed (Navas & Jimenez, 1996, 1999). However, chemiluminescence methods require high-level techniques, such as column and solvent selection and emitter identification, due to (1) poor reproducibility by the second oxidation of emission products, (2) emission change by energy transfer between the emitter and fluorescence impurities and (3) self-absorption and the disappearance of emission during long observation times. We have been developing imaging quantification using a charge-coupled device (CCD) camera to overcome some disadvantages of chemiluminescence analysis (Yoshiki, Iida, Akiyama, Okubo, Matsumoto, & Sato, 2001, Yoshiki, Kanazawa, & Okubo, 2002). Photon detection, under the $KHCO_3$/MeCHO conditions is an easy system for the quantification of hydroperoxide and hydrogen donor. For instance, the tea polyphenols studied in this paper are detected selectively as hydrogen donors in the H_2O_2/$KHCO_3$/MeCHO chemiluminescence system. The advantages of our chemiluminescence system as an evaluation system for food are (1) short measuring time (10 min), (2) simultaneous measurement of 10–20 samples, (3) applicability to liquid and solid samples and (4) simplified measurement technique.

The aim of this report is to investigate the utility of the photon detection system in food evaluation. We

Abbreviations: H_2O_2: hydrogen peroxide; O_2^-: superoxide; DPPH: 1,1-diphenyl-2-picrylhydrazyl; MeCHO: acetaldehyde; CCD camera: charge-coupled device camera.

* Corresponding author. Tel.: +81-22-717-8825; fax: +81-22-717-8829.
E-mail address: yoshiki@bios.tohoku.ac.jp (Y. Yoshiki).

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have studied the emission properties of several kinds of tea: green tea, oolong tea and black tea, and compared them with polyphenol contents and chemopreventive activities against H_2O_2, O_2^- and DPPH radical. We also examine the utility of this photon detection system in the manufacturing process of green tea.

2. Materials and methods

2.1. Reagents

Gallic acid and $KHCO_3$ were obtained from Nakarai Tesque (Kyoto, Japan). Hydrogen peroxide (30%), 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH radical) and acetaldehyde (MeCHO) were purchased from Santoku Chem. (Tokyo, Japan), Tokyo Kasei (Tokyo, Japan) and Merck (Darmstadt, Germany), respectively. (−)-Epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate and (−)-epigallocatechin gallate, from *Camellia sinensis*, were obtained from Kurita Ltd. (Tokyo, Japan).

2.2. Materials

Fifteen kinds of commercial tea, green tea, oolong tea and black tea were infused with distilled water (0.5 g/20 ml) overnight. To study photon emission in the manufacturing process of green tea, tea leaves were used at each manufacturing step: raw leaves (nama-ba), steamed leaves (mushi-ba), primary rolling leaves (sojyu-cha), secondary rolling leaves (cyujyu-cha), crude tea (ara-cha) and green tea (sen-cha). Each leaf was freeze-dried and extracted with distilled water (50 mg/ml) for 1 h. Tea infusion was used for the following examination.

2.3. Detection of photon emission

Chemiluminescence was detected with a charge-coupled device (CCD) camera (Hamamatsu Photonics, Japan). The CCD camera was connected to an imaging PMP (photocathode microchannel plates) and a position-sensitive detector coupled with an ARGUS-20 image processor for further image enhancement and quantitative analysis. The wavelength range of the detector was 350–850 nm, 512×483 pixels. The reaction mixture contained 196 mM H_2O_2 (0.5 ml), 2.5 mg/ml tea infusion (0.5 ml) and 100 mM $KHCO_3$ in 356 mM MeCHO (0.5 ml). The reaction solution was added in a 12-hole multi-plate (∅12 mm; total volume; 1.5 ml). Photon intensity was given by relative luminance (cd/m^2). Emission spectra in the visual region were measured with a Simultaneous Multiwavelength Analyzer model CLA-SP2 (Tohoku Electronic, Japan). The wavelength range of the spectroscope was 400–850 nm. Light emission was determined for 180 s.

2.4. Determination of total phenolic compounds

The total phenolic compounds were determined spectrophotometrically, using Folin–Ciocalteu's phenol reagent (Sigma, St. Louis, MO). The Folin–Ciocalteu's phenol reagent (150 μl) was added to the tea infusion (50 μl). After 3 min, 1 g of Na_2CO_3 and 4.8 ml of distilled water were added to the reaction mixture. The mixture was incubated at 37 °C for 1 h, and the blue colour at 680 nm was measured with a spectrophotometer (Shimazu UV-1600) (Yen & Duh, 1994). The total phenolic content was determined by comparison with the absorption from the standard curve of gallic acid. The total phenolic compound contents of tea were converted into gallic acid equivalents (nmol).

2.5. Determination of catechin contents

Catechin contents of tea were determined by HPLC analysis. Samples (10 μl) were analyzed on a reversed-phase column (YMC ODS AM-303, 250×4.6 mm ID, particle size 5 μm). The mobile phase was methanol-water-phosphoric acid (300:700:1). The flow rate was 0.9 ml/min and the analysis wavelength was 210 nm (Dalluge & Nelson, 2000).

2.6. H_2O_2 scavenging activity

Hydrogen peroxide (0.25 ml of 7.84 mM H_2O_2) was measured by allowing the sample solution (0.1–0.5 ml), 3.6 M $KHCO_3$ in 356 mM MeCHO (0.3 ml) and water (0.95–0.55 ml) to react with 0.25 ml of 20% (w/v) H_2SO_4 and 0.15 ml of 1 M $TiSO_4$. The hydrogen peroxide (H_2O_2) concentration was determined by absorption at 408 nm (Shimazu UV-1600) and calculated using the H_2O_2 standard curve.

2.7. DPPH radical scavenging activity

An ethanolic solution of DPPH radical (final concentration of DPPH was 2.0×10^{-4} M) was added to 0.1 M acetic acid buffer (pH 5.5, 1 ml) and a sample solution (1 ml) mixture. The mixture was shaken and left to stand for 10 min. The colour at 517 nm was measured with a spectrophotometer (Shimazu UV-1600) (Yen & Duh, 1994).

2.8. O_2^- scavenging activity

Superoxide (O_2^-) scavenging activities were measured using the ESR spin-trapping method. ESR spectra were recorded on a JEOL JES-RE1X Spectrometer (JEOL Ltd., Japan) using an aqueous quartz flat cell (60 mm × 10 mm × 0.31 mm, effective volume 160 μl), xanthine oxidase (0.4 U/ml, 50 μl) and DMPO (9.2 mol/l, 20 μl) for the purpose of causing O_2^- scavenging activity.

The O_2^- scavenging activity was calculated using the DMPO-O_2 spin adduct index and was compared with that of SOD (Mitsuta, Mizuta, Kohno, Hiramatsu, & Mori, 1990).

3. Results and discussion

3.1. Photon emission of tea

The three most popular tea types in the world are green tea, oolong tea and black tea. Photon emissions of the three commercialized teas were investigated in the H_2O_2/$KHCO_3$/MeCHO system (Fig. 1). Each tea ($n = 5$) produced photon emission in the visual region at 610–640 nm (green tea), 630–650 nm (oolong tea) and 640–670 nm (black tea). In the same emission system, gallic acid has an emission maximum at 650 nm and catechin at 610 nm. Emission spectral analysis suggested that the emission source of tea resulted in phenolic compounds. The order of photon intensity was: green tea ($1202 \pm$ SD 173.51 cd/m^2), oolong tea ($834 \pm$ SD 237.44 cd/m^2) and black tea ($222 \pm$ SD 87.65 cd/m^2). Since photon emission from tea was a reaction with a very high velocity (green tea, 6×10^5 counts/s), we needed the concentration set, for an accurate measurement for the imaging detection of photon emission, using the CCD camera. In the 196 mM H_2O_2 (0.5 ml) and 100 mM $KHCO_3$/356 mM MeCHO (0.5 ml) system, 2.5 mg/ml (0.5 ml) of tea infusion was the optimum concentration for photon detection.

3.2. Polyphenol and catechin contents

The three tea types are distinguished by their contents of methylxanthines and polyphenols, especially flavonols (catechin). We studied the relationship between photon emission-polyphenol and photon emission-catechin. Polyphenol analysis of tea with the spectrophotometer showed $3.22 \pm$ SD 0.39 μmol/mg for green tea, $2.37 \pm$ SD 0.40 μmol/mg for oolong tea and $1.49 \pm$ SD 0.43 nmol/mg for black tea (gallic acid conversion).

The major tea polyphenols were catechin, (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (+)-gallocatechin (GC) and (+)-catechin (Arts, Rutte de van, & Hollman, 2000). HPLC, with UV absorbance detection, could identify four catechins, EGC (Rt. 4.68 min), EGCG (Rt. 14.67 min), EC (Rt. 16.92 min) and ECG (Rt. 39.0 min) (Fig. 2). However, in the tea infusion analysis, the EGC peak could not be distinguished from the void fraction peak of tea due to an early retention time. Tea catechin contents were given by the total amounts of EGCG, EC and ECG. Green tea contained catechin at $2.66 \pm$ SD 0.51 μmol/mg, oolong tea was at $1.24 \pm$ SD 0.57 μmol/mg, and black tea was at $0.67 \pm$ SD 0.51 μmol/mg. Tea catechin accounts for 79.0% (green tea), 49.4% (oolong tea) and 39.4% (black tea) of polyphenol contents. Balentine (1991) reported that about 80% of catechins were biochemically oxidized in the black tea. The catechin content analysis (oolong tea/green tea $\times 100 = 40\%$, black tea/green tea $\times 100 = 25\%$) supported their result, while oolong tea and black

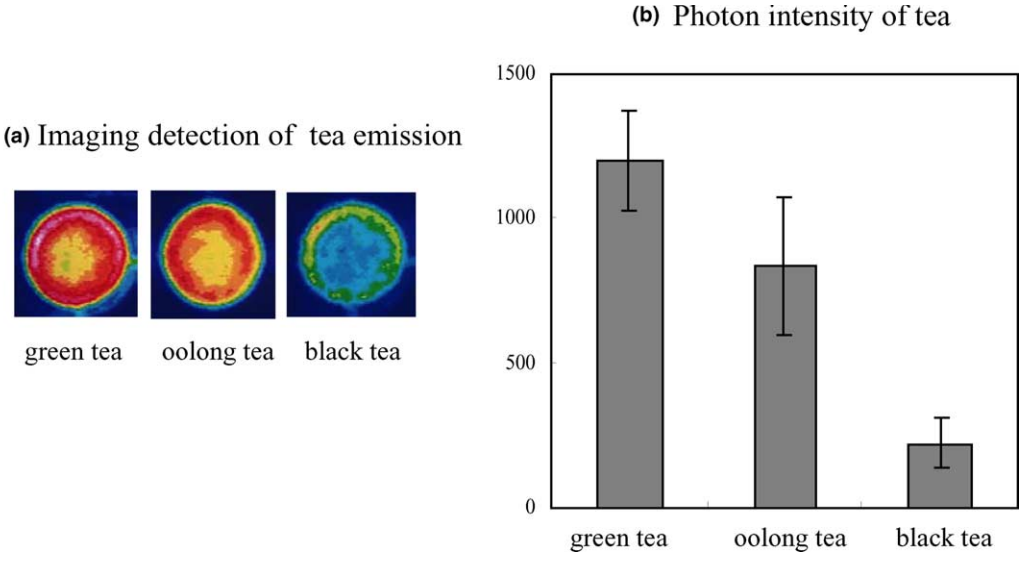

Fig. 1. Photon emission of tea in the H_2O_2/$KHCO_3$/MeCHO system. (a) Imaging detection of photon emission from tea using CCD camera. Tea infusion was 0.05 g/20 ml. (b) Photon intensity from 0.5 ml of tea infusion (0.05 g/20 ml) in the presence of 196 mM H_2O_2 (0.5 ml) and 100 M $KHCO_3$/356 mM MeCHO (0.5 ml). Values are 5 samples averaged, and vertical bars represent SD.

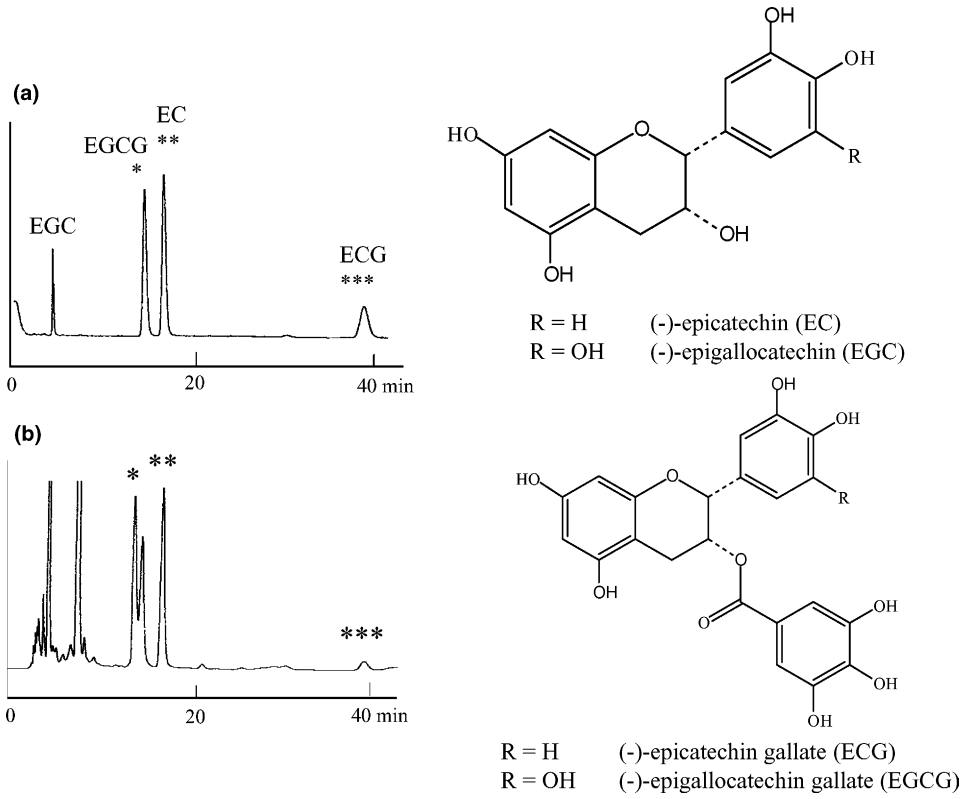

Fig. 2. HPLC elution patterns and structures of major catechins in tea. (a) HPLC separation of a catechin standard mixture. The concentrations of the four compounds were: 0.6 nM (−)-gallocatechin (GC), 33.7 nM (−)-epigallocatechin gallate (EGCG), 15.2 nM (−)-epicatechin gallate (EGC) and 69.7 nM (−)-epicatechin (EC). (b) HPLC separation of green tea infusion (0.5 mg/20 ml). Injection volume is 10 μl.

tea contained polyphenol at 74% and 48% of green tea, respectively. Photon intensity of tea showed a high correlation with the total polyphenol contents ($r^2 = 0.881$) rather than with the catechin contents ($r^2 = 0.7759$) (Fig. 3).

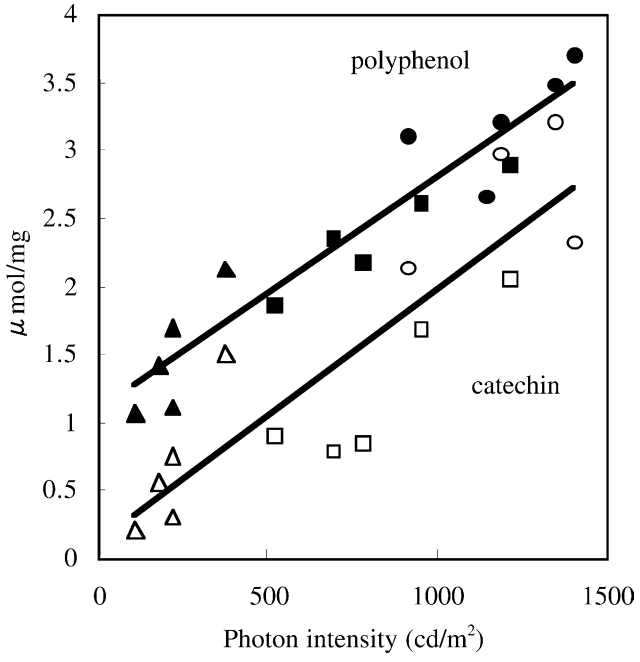

Fig. 3. Relationship photon intensity-polyphenol and -catechin. Polyphenol, filled symbol; catechin, open symbol; green tea, circles; oolong tea, squares; and black tea, triangles.

3.3. Chemopreventive activities against O_2^-, DPPH radical and H_2O_2

It is well known that tea infusion and its individual components, especially polyphenols, have antimutagenic, antigenotoxic and anticlastogenic effects (Kada, Kaneko, Matsuzaki, Matsuzaki, & Hara, 1985; Hasegawa, Chujo, Saikato, Umemura, Tanimura, & Kurokawa, 1995; Yang, Chung, Yang, Chhabra, & Lee, 2000). Such protective action of tea polyphenols seems to result from their known antioxidant properties and reactive oxygen scavenging activities (Sarkar & Bhaduri, 2001). We investigated O_2^-, DPPH radical and H_2O_2 scavenging activities of tea infusion and compared them with photon intensity. Superoxide (O_2^-) and DPPH radical scavenging activities of tea were $20.22 \pm$ SD 8.86 SOD U/50 mg and $81.43 \pm$ SD 11.59 nmol/10 mg (green tea), $16.05 \pm$ SD 7.07 SOD U/50 mg and $59.03 \pm$ SD 12.31 nmol/10 mg (oolong tea), and $2.63 \pm$ SD 3.52 SOD U/50 mg and $35.63 \pm$ SD 8.52 nmol/10 mg (black tea). A higher correlation of photon emission was observed with O_2^- ($r^2 = 0.7998$) than with DPPH radical scavenging activity ($r^2 = 0.7516$) (Fig. 4).

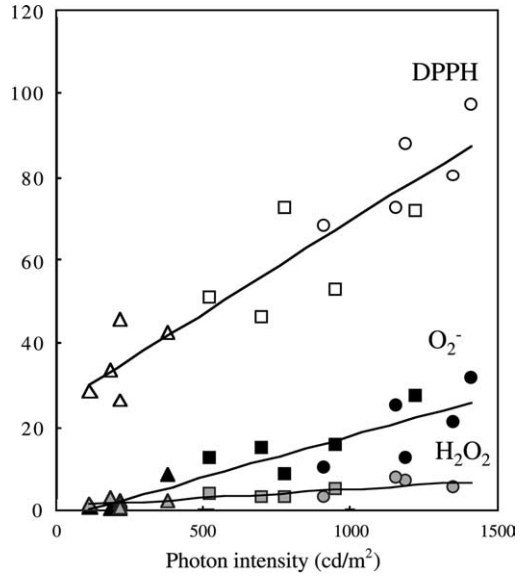

Fig. 4. Relationship between photon intensity and chemopreventive activity against H_2O_2, O_2^- and DPPH radical. DPPH radical scavenging activity, open symbol; O_2^- scavenging activity, filled symbol; H_2O_2 scavenging activity, gray symbol; green tea, circles; oolong tea, squares; and black tea, triangles. The vertical axis represents nmol/10 mg for DPPH radical, SOD U/50 mg for O_2^- and mmol/5mg for H_2O_2 scavenging activities. H_2O_2 scavenging activity of tea infusion was measured in the presence of 0.54 M $KHCO_3$ and 53 mM MeCHO.

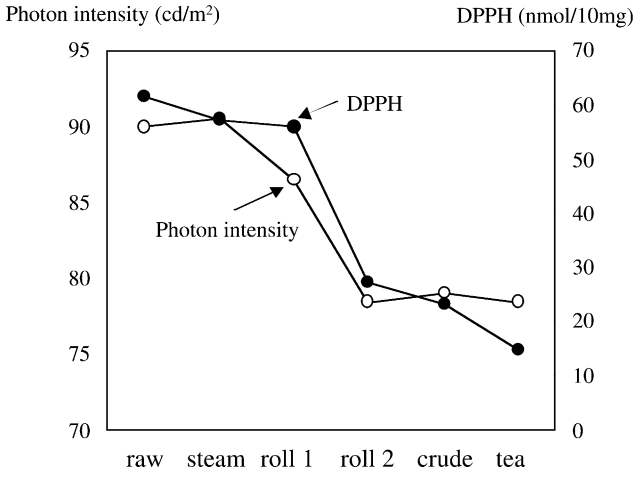

Fig. 5. Photon intensity and DPPH radical scavenging activity on manufacturing process of green tea DPPH radical scavenging activity, filled circles; photon intensity, open circles. raw, raw leaves: steam, steamed leaves; roll 1, primary rolling leaves; roll 2, secondary rolling leaves; crude, crude tea; and tea, green tea.

Recently, we reported unique H_2O_2 scavenging activity of gallic acid in the chemiluminescence system (H_2O_2/gallic acid/$KHCO_3$/MeCHO system) (Yoshiki et al., 2001). Tea infusion also show H_2O_2 scavenging activity in the presence of $KHCO_3$ and MeCHO, while tea infusion without $KHCO_3$ and MeCHO did not showed H_2O_2 scavenging activity. Fig. 4 shows H_2O_2 decrease by tea infusion in the presence of 0.54 M $KHCO_3$ and 53 mM MeCHO (final concentration). Fifty microlitre of tea infusion (0.5 g /20 ml) decreased H_2O_2 to $1.21 \pm$ SD 0.41 mmol/5 mg for green tea, $0.77 \pm$ SD 0.17 mmol/5 mg for oolong tea and $0.42 \pm$ SD 0.16 mmol/5 mg for black tea. Hydrogen peroxide (H_2O_2) scavenging activity also showed a correlation with photon intensity ($r^2 = 0.7516$).

3.4. Photon emission on the manufacturing process of green tea

Although the major cause of oxidized polyphenol in fresh tea leaves is biochemical oxidation by polyphenol oxidase, polyphenols are partly oxidized in the manufacturing process (Arts et al., 2000). If we can make an assessment of the polyphenol contents, oxidation ratio and chemopreventive potential through a simple photon detection technique, it is possible to control the production line to maintain the chemopreventive and antimutagenic effects in fresh tea leaves. We studied the utility of this photon detection system in the manufacturing process of green tea. Tea leaves were obtained from each manufacturing step, raw leaves (raw), steamed leaves (steam), primary rolling leaves (roll 1), secondary rolling leaves (roll 2), crude tea (crude) and green tea (tea) (Fig. 5). A marked difference in quality of raw leaves was observed in the secondary rolling step. Photon intensity and DPPH radical scavenging activity of the secondary rolling leaves showed 20% and 75% decrease compared with that of raw leaves. The difference between the primary rolling leaves step and the secondary rolling leaves step was due to the uniform change of moisture with pressure. Emission analysis in the manufacturing process of green tea suggested that the pressure process rather than the heat process, in the tea production line, causes a loss in the chemopreventive potential of polyphenol.

4. Conclusions

The present study, using commercial tea, green tea, oolong tea and black tea, showed that (1) photon intensity of tea in the H_2O_2/$KHCO_3$/MeCHO system has a high correlation with polyphenol contents rather than catechin contents; (2) chemopreventive activities of tea against H_2O_2, O_2^- and DPPH radical could be estimated by photon intensity, due to the high correlation between photon intensity and these activities; (3) photon intensity reflected the chemopreventive potential of tea and/or polyphenol in the manufacturing process. Photon emission from tea in the H_2O_2/$KHCO_3$/MeCHO system could contribute to a better understanding of the tea quality responsible for these chemopreventive effects. The imaging detection (visual technique) of the photon

emission of tea, shown in Fig. 1, might be more useful for tea evaluation. In addition, this method has an advantage in its application compared to other techniques in the manufacturing process because it can measure 10–20 samples simultaneously in 10 min.

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