Effects of Ultraviolet Radiation on the Color of Compounded Papers Containing Wasted Tea Leaves

Tetsuya Takahashi^{*1}, *Wakako Kasai*^{*2}, and *Tetsuo Kondo*^{*2}

^{*1} Faculty of Education, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan

^{*2} Bio-Architecture Center, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

Abstract: Influence of ultraviolet irradiation on papers containing wasted tea leaves was studied. Ultraviolet irradiation generally caused reddish coloration. Irradiation under dry atmosphere caused stronger coloration than under ambient conditions. Presence of oxygen did not cause significant difference in coloration. Thin-layer chromatographic analysis showed disappearance of chlorophylls a and b, and did not disappearance of theaflavin by UV irradiation. The results strongly suggest that the coloration is caused by photochemical conversions of catechins to reactants (theaflavin-like compounds).

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1. Introduction

Tea has been widely consumed as a favorite healthy drink for a long time because it has a characteristic refreshing aroma and a rich taste. Tea has been deep-rooted in the eating habits and is also popular in Japan [1]. The recent tea boom has encouraged the usage of PET-bottled and canned tea, which anyone can easily access anytime [2]. A negative side of the boom is that approximately 100,000 metric tons of wasted tea leaves are dumped as industrial waste [3], which demands effective treatment methods.

Hence, an attempt was made to take advantage of catechins present in wasted tea leaves for the development of functional papers [4-6]. These are assumed to be for applications to interior goods, such as wall paper. The papers were demonstrated to possess characteristics such as antibacterial activity and deodorant ability [4,6]. Further, ultraviolet (UV) irradiation increased the antibacterial activity of the papers [7]. However, the UV treatment discolored the papers. Therefore, this study attempted to elucidate the mechanism underlying the change in the hue of papers containing wasted tea leaves.

2. Experimental

2.1 Samples

2.1.1 Preparation of wasted tea leaves

The study involved the following 5 kinds of tea leaves : green tea (Sencha), oolong tea, black tea, pu-erh

tea, and hoji-cha [4]. These tea leaves are fermented to different levels by different methods. Green tea leaves were obtained from ITO EN, LTD. ; oolong tea leaves, from Nihon-cha Hanbai K.K. ; black tea leaves, from Mitsui Norin Co., Ltd. ; hoji-cha leaves, from Nihon-cha Hanbai K.K. ; and pu-erh tea leaves, from TENJIN SEICHA [4].

A prescribed amount of tea leaves was added to a specific volume of boiling distilled water in an enamel kettle and boiled for 30 min. A fine stainless steel mesh ball was used to filter the liquid. To 4.0 ℓ of distilled water, 400 g of green tea (Sencha) leaves was added and the mixture was heated to 70°C for extraction of tea. In the case of oolong and black teas, heating temperature was set to 90°C for the extraction. For hoji-cha, 300 g of tea leaves was added to 3.0 ℓ of distilled water, and the mixture was heated to 90°C for the extraction. In the case of pu-erh tea, 250 g of tea leaves in 2.5 ℓ of distilled water was heated to 90°C for the extraction.

2.1.2 Preparation of papers containing wasted tea leaves for irradiation experiments

Papers containing wasted tea leaves were prepared by the method reported previously [4-6]. Briefly, the wasted tea leaves were ground under wet conditions with a Mass-Colloider (Stone mill type crusher) with a clearance of 40 μm, before blending with desired amounts of pulp and latex binder. The leaf content was adjusted to 60 wt% in the formulation whereas the latex binder content was 0.3 wt% of the total amount of the wasted tea leaves and the pulp in all the formulations. The pulp used was prepared by refining Canadian bleached conifer kraft

pulp to a standard freeness of 550 ml with a refiner. A latex binder (Aica Aibon RAX117 from Aica Kogyo Co., Ltd.) made of styrene-butadiene rubber (SBR) was used to improve the adhesion between pulp and wasted tea leaves. The binder is mainly used for paper processing, which commonly involves strengthening and coating of papers. After distilled water was added to the above mixtures, they were stirred with a mixer for 10 s to obtain slurries of homogeneous dispersion. An angular-sheeting machine (PU-401) from Tester Sangyo Co., Ltd., was used to adjust the slurries to a paper weight of 100 g/m² in accordance with JIS-P8209 and to prepare paper sheets of size 25 × 25 cm. The sheets were pressed with a pressure of 410 kPa at room temperature and dried at ca. 120°C with a rotating drier to finally obtain papers containing wasted tea leaves for irradiation treatment as follows.

SUNTEST CPS, a xenon tester [8,9] from Shimadzu Corporation, was used to irradiate the prepared papers for 1–200 h [7]. A digital UV detector, UVX, from Ultraviolet Products, LLC, was used to measure the respective irradiation intensities of 9,260 µW/cm² at 365 nm, 8,420 µW/cm² at 310 nm, and 1,291 µW/cm² at 254 nm. The values were converted into a cumulative UV dose per hour of 3.33×10^5 J/m² at 365 nm, 3.03×10^5 J/m² at 310 nm, and 4.65×10^4 J/m² at 254 nm. The atmospheric temperature in the irradiation chamber was 38.4°C during the irradiation. In order to investigate the effect of wavelength on color change, colored glass filters from Asahi Techno Glass Corporation were used to cover the paper during irradiation.

2.2 Measurements

2.2.1 Spectrophotometry

A spectrophotometer (UV-3100; Shimadzu Corporation) was used to measure the reflectance spectra of the irradiated paper samples at 450–800 nm on standard white plates of barium sulfate. The slit width was 2.0 nm and the sampling pitch was 0.5 nm. A value of 0 was assigned to the absorbance of the white plate as the standard calibration.

The photo-induced changes in the hue of the papers containing wasted tea leaves seem to be affected by moisture and oxygen in the atmosphere. Thus, a gas together with a paper containing wasted tea leaves was introduced into a bag made of polyvinyl fluoride (Tedlar®), which is a good oxygen barrier, and the bag was irradiated with UV light. The transmittance spectrum of the film used to produce the bag was measured before the experiment. The film had a transmittance of 78.6% at a wavelength of 365 nm, 69.1% at 310 nm, and 31.8% at 254 nm. The film also had a peak of a high transmittance

at approximately 255 nm. Thus, the bag was found to absorb most rays of wavelengths less than 290 nm and to allow rays in the UV region to pass through relatively well.

Thus, papers containing wasted tea leaves were placed in a bag with air containing oxygen or in a bag with pure nitrogen gas without oxygen. The bags of the 2 test groups were irradiated with UV light to examine the effect of atmospheric oxygen on UV irradiation. In the experiment, each test group comprised 2 sub-groups: in 1 sub-group, a desiccant was added in the bag along with the paper sample, and the bag was left standing for 24 h to allow it to reach a dry state before UV irradiation; in the other sub-group, a desiccant was not added and the experiment was performed under ambient conditions. This test allowed the examination of the effect of atmospheric moisture on the changes caused by UV irradiation. The 4 sub-groups were irradiated with UV light for 1 h or 20 h.

2.2.2 Pigment separation using thin-layer chromatography (TLC)

Fat-soluble pigments were extracted with diethyl ether (Kanto Chemical Co. Inc.) and separated on silica gel TLC plates (Tokyo Kasei Kogyo Co. Ltd.). To petroleum ether, acetone was added to form a mixed solution (8 : 2), which was used as the developing solvent. Then, diethyl ether was added to wasted tea leaves ground using mortar and pestle. A few drops of the supernatant containing the pigments were applied to form a densely colored spot 2 cm above the bottom end of a TLC plate. The development was completed in 25 min after the developing solution began to ascend. After drying the TLC plates, the distance (L) that the developing solution traveled and the distances (X₁, X₂, X₂', X₃, and X₃') the pigments migrated were measured to calculate the rates of flow (R_f values). The equation to determine R_f values is given below.

$$R_f = X_1/L, X_2/L, X_2'/L, X_3/L, X_3'/L$$

3. Results and Discussion

3.1 Dependency of the hue of the papers containing wasted tea leaves on UV irradiation

The 5 kinds of irradiated paper samples (green tea, oolong tea, black tea, hoji-cha, and pu-erh tea) clearly showed coloring, as shown in Fig. 1. The photographs show that the reddish hue of the non-irradiated papers containing wasted tea leaves increased with increase in the fermentation level of the teas from green tea through oolong tea up to black tea. When the papers were

Irradiation time	Green tea	Oolong tea	Black tea	Hoji-Cha	Pu-Erh tea	Pulp
Before
1.0 hour
20 hours

Fig. 1 Papers containing 60wt% of wasted tea leaves after UV irradiation.

irradiated with UV light for 1 h, all the papers except the paper containing wasted green tea leaves were reddish. After 20 h of UV irradiation, the reddish brown color of all the papers became more intense.

Hence, to quantitatively analyze the abovementioned changes in the color of these papers, the absorbance spectra of the papers was measured using a spectrophotometer. Figure 2 exhibits the spectra of 2 paper samples containing 60 wt% wasted green tea leaves (a) and 60 wt% wasted black tea leaves (b). UV irradiation was clearly shown to lower the absorbance peak near 675 nm observed with the non-irradiated paper containing wasted green tea leaves. Conversely, a 500–550 nm absorption band for green, the color complementary to red, increased fairly with increase in the UV irradiation time. In other words, UV irradiation was quantitatively shown to turn the hue of the paper containing wasted green tea leaves increasingly reddish (Fig. 2(a)). When the paper containing wasted black tea leaves was irradiated with UV light, changes in absorbance were relatively small (Fig. 2(b)). The originally intense reddish pigment of the paper containing wasted black tea leaves seemed to mask the hue changes caused by UV light.

3.2 Effect of atmospheric conditions on the changes due to UV irradiation

A spectrophotometer was used to analyze hue changes of the papers containing wasted tea leaves. Reflected light was measured to determine the absorbance of these papers at 450–800 nm, which is the range of wavelength for the visible region. Figure 3 shows the absorbance of the paper containing wasted green tea leaves that was irradiated with UV light for 1 h (a) or 20 h

(b). Consequently, UV irradiation was found to shrink an absorption peak observed before UV irradiation near 675 nm, which corresponds to the wavelength for red color. Furthermore, UV irradiation for 20 h (b) resulted in the shrinking of the absorption peak near 675 nm to a greater extent than that by UV irradiation for 1 h (a). The shrinking of the absorbance peak was slightly less in the

Fig. 2 Absorbance of papers versus wavelength after UV irradiation.
(a) Papers containing 60wt% of wasted green tea leaves (b) Papers containing 60wt% of wasted black tea leaves

Fig. 3 Absorbance of papers containing 60wt% of wasted green tea leaves versus wavelength for different condition in the visible region.
(a) After UV irradiation of 1.0 hour
(b) After UV irradiation of 20 hour

ambient state than in the dry state in the presence of air or nitrogen gas. This phenomenon seems to be due to the absorption of UV light by atmospheric moisture, which decreases the effect of UV light energy.

Absorbance by the paper containing wasted green tea leaves also tended to be lower on UV light irradiation in the atmosphere containing oxygen than on irradiation in the atmosphere of nitrogen gas. This can be attributed to the oxygen in the air, which readily causes the oxidative polymerization of catechins to form catechin reactants (theaflavin-like compounds) on irradiation of the paper with UV light in the air. However, the difference was very small; the hue of the paper changed to reddish brown even when irradiated with UV light in the absence of oxygen. The development of the reddish brown pigment seems to be because of chemical changes associated with radical production by UV irradiation. The chemical changes are believed to be chemical reactions that differ from the oxidative polymerization catalyzed by polyphenol oxidase [10], a well-known oxidase.

3.3 Separation of pigments extracted from wasted tea leaves by TLC

As described above, UV irradiation was found to markedly change the hue of papers containing wasted tea

leaves. In order to understand the details of the hue changes of the papers by UV light, the separation of ingredients contained in wasted tea leaves was attempted. TLC plates were used to separate pigments extracted from all wasted tea leaf samples (green tea, oolong tea, black tea, pu-erh tea, and hoji-cha) before UV irradiation.

Figure 4 shows the UV-irradiated pigments separated by TLC plates; Table 1 shows the flow (Rf values) for pigments from the various kinds of wasted tea leaves before UV irradiation. The TLC plate before UV irradiation exhibited spots corresponding to chlorophyll a (spot 3), its decomposed product or pheophytin a (spot 3'), chlorophyll b (spot 2), and its decomposed product or pheophytin b (spot 2'). Although spots varied in color density depending on the samples, the theaflavin (spot 1) pigment was identified at the initial position where the sample solution was applied.

UV irradiation for 0.3 h was found to entirely fade the spots for all the pigments, and eventually, 1 h of irradiation erased most pigment spots except 2 spots: those for chlorophyll a and theaflavin. After 20 h of UV irradiation, only spots due to the theaflavin pigment remained at the initial position on the TLC plate. Spots of the theaflavin pigment could be observed even after further UV irradiation up to 100 h. This indicates that theaflavins in the 5 samples are relatively stable and are not decomposed by UV irradiation. It is necessary to determine components remaining at the starting point on TLC. This will be an issue to be worked on in the future."

In order to quantitatively analyze changes in color density, an image analysis software (Image J) was used and the densities of the pigment spots were measured from photographs of the TLC plates. Figure 5 shows the changes in the color density of spots corresponding to pigments from wasted green tea leaves. Pigments such as chlorophyll a and chlorophyll b completely disappeared after 20 h of UV irradiation. In contrast, theaflavin pigments were quantitatively found to remain stable even after longer periods of UV irradiation. Therefore, the reddish color of irradiated papers, as shown in Fig. 1, may be due to the reddish color of catechin reactants (theaflavin-like compounds).

It is also necessary to determine components of the residue from extraction with diethyl ether. This will be an issue to be worked on in the future.

3.4 Changes in the hue of theaflavin and catechin upon UV irradiation

In the previous section, it was assumed that the color of catechin reactants (theaflavin-like compounds) may be the major hue of the irradiated paper samples containing

Fig. 4 The changes of wasted tea leaves on silica gel Thin-Layer Chromatography template by UV irradiation (Solvent for extraction : Diethyl ether, Solvent for separation : Petroleum Ether and Acetone mixtures (8 : 2), Time for separation : 25 minutes). ① Green tea, ② Oolong tea, ③ Black tea, ④ Hoji-Cha, ⑤ Pu-Erh tea
1 : Theaflavin, 2 : Chlorophile b, 2' : Pheophytin b, 3 : Chlorophile a, 3' : Pheophytin a

wasted tea leaves. Thus, an attempt was made in this section to examine how UV light changes the hues of theaflavin as well as various catechins. In this examination, KBr pellets containing the following 5 kinds of catechin compounds were prepared : epigallocatechin gallate ((-)-EGCg), epigallocatechin ((-)-EGC), epicatechin gallate ((-)-ECg), epicatechin ((-)-EC), and catechin ((+)-C). The pellets were then irradiated with UV light to cause the apparent color changes, as shown in Fig. 6. The results showed that the color of theaflavin 3-*O*-gallate (TF2A), as a representative of theaflavin, did not change with UV light irradiation, which was expected.

With regard to the catechin compounds, (-)-EGCg, the most abundant catechin in green tea, was only slightly reddish after 1 h of UV irradiation. Then, it turned clearly reddish after 5 h of UV irradiation, and its reddishness further increased in intensity until 20 h of UV irradiation. Other catechin compounds such as (-)-EGC, (-)-ECg,

Table 1 Rate of flow (Rf value) of various wasted tea leaves on Thin Layer Chromatography.

	Green tea	Oolong tea	Black tea	Hoji-Cha	Pu-Erh tea
Theaflavins	0	0	0	0	0
Pheophytin <i>a</i>	0.716	0.686	—	—	—
Chlorophyll <i>a</i>	0.649	0.635	0.635	0.695	0.681
Pheophytin <i>b</i>	0.525	—	—	0.511	0.563
Chlorophyll <i>b</i>	0.461	0.513	0.483	0.468	0.517

Rate of flow (Rf Value) =

Transfer Distance of Solute / Transfer Distance of Solvent = a/h

Fig. 5 Thin Layer Chromatographic determination of wasted green tea leaves after UV irradiation.

and (-)-EC also showed a similar tendency. However, the change in the color of (-)-ECg to a reddish hue was fairly lesser than in the cases of the above catechins, and instead, (-)-ECg turned yellowish upon UV irradiation.

Considering the above results, the colored catechin reactants (theaflavin-like compounds) possibly contribute to the hues of the irradiated paper samples containing wasted tea leaves.

4. Conclusions

This study showed that UV irradiation of the compounded papers converted catechins present in the

	(-)-EGCg	(-)-EGC	(-)-ECg	(-)-EC	(+)-C	Ref.) TF2A
Before
1.0 h
5 h
10 h
20 h

Fig. 6 Colour changes of Catechins in KBr pellets by UV irradiation.

wasted tea leaves to their reactants (theaflavin-like compounds) and stabilized the color of the papers. In addition, the conversion to catechin reactants can increase the antimicrobial potency of compounded papers containing wasted tea leaves [7]. For example, stable color hue is an important requirement for wallpapers. The additional advantage of increased antimicrobial potency is also very desirable. As described above, compounded papers containing wasted tea leaves are very promising not only for the effective utilization of wasted tea leaves but also as excellent functional paper with stable color hue.

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