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Simple Constituent Analysis and Quality Evaluation Criteria for Preventing Deterioration of Powdered Green Tea

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Abstract: Heat treatment in the green tea manufacturing process converts chlorophyll (Chls: green color) contained in raw tea leaves into pheophytins (Pheos: gray color), which has a significant impact on the appearance quality of the product. In this study, to quantitatively evaluate this thermal discoloration behavior, Chl-a and Pheo-a were separated and analyzed using circular paper chromatography (CPC), which is an omnidirectional diffusion type with a wide separation band suitable for spectroscopic analysis. Using the conversion ratio (Pheo-a/Chl-a) calculated by spectroscopic analysis as an indicator, it was shown that the conversion ratio when boiling fresh tea leaves differed significantly depending on the harvest time and plucking order, and that an increase in this value is a direct factor that promotes the gray-greening of tea leaves. Furthermore, because the discoloration characteristics of commercially available green tea vary depending on the processing method, blending, and the presence or absence of additives, the importance of individual evaluation taking into account the labeled ingredients was confirmed. The evaluation method based on the conversion rate established in this study is expected to contribute to the optimization of the heating process of green tea, quality control, and as an objective indicator for the formulation of quality guidelines for tea leaves.

Key words: Green tea, chlorophyll, pheophytin, fading, conversion rate.

1. Introduction

The quality of green tea is primarily judged by its vibrant green color and glossy appearance, which are widely regarded as indicators of both freshness and flavor profile [1, 2]. During the manufacturing and storage processes, however, chlorophylls (Chls)—the pigments responsible for this characteristic green—are highly susceptible to degradation. Exposure to moisture, heat, ultraviolet light, and acidic conditions causes Chls to lose their central magnesium ion, converting them into gray-green pheophytins (Pheos) [3, 4]. Furthermore, enzymatic pathways involving chlorophyllase and pheophorbidase can lead to the formation of chlorophyllides and pheophorbides, further contributing to color deterioration [5].

In the commercial production of green tea, discoloration is driven by several critical factors: enzymatic degradation following harvest, thermal stress during steaming, rolling, and drying, and high humidity levels post-manufacturing. While specialized processes such as cold transportation and optimized drying stages are essential for developing flavor and maintaining quality [6], these heating phases inevitably impact the tea's final appearance. Despite its importance, the objective color evaluation of commercially available tea blends remains a challenge due to the complexity of their compositions [7].

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While sensory qualities such as taste and aroma are integral to quality assessment, their analysis is often highly specialized, diverse, and technically demanding [8-10]. Consequently, there is a practical need for a more accessible yet reliable method to evaluate quality based on pigment stability. This study, therefore, developed a simplified analytical approach to monitor Chls and Pheos, which serve as key determinants of both color and flavor. Using this method, we investigated the conversion behavior of Chl-a to Pheo-a during the thermal processing of fresh leaves and evaluated the conversion rates across various commercial green tea powders to establish more practical quality evaluation criteria.

2. Experimental Method

Chl-a is converted into Pheo-a, a process closely linked to the discoloration of green tea. Paper chromatography (PC) was employed for the simplified analysis of both components [11].

2.1 Analysis of Chls and Pheos Components

To investigate the discoloration and degradation of powdered green tea, Chl-a, and Pheo-a other constituents were separated using circular paper chromatography (CPC). The absorbance of each separated band was subsequently measured. All procedures were conducted in the dark to prevent photodegradation of the pigments.

2.2 Sample Breakdown

a) Fresh tea leaves (cv. Yabukita, Ecoro Farm, Yamanashi Prefecture) harvested during the 2025 season were used as samples. These included the first-flush (May 12), second-flush (June 16), and late-season tea (autumn/winter tea, harvested on or after September 26). Samples were transported under refrigeration on the day of the experiment and used immediately.

b) The following commercial powdered green tea products were purchased in 2025:

Powdered tea leaves: Six products, consisting of three additive-free products, one organic product, and two products containing secondary ingredients such as matcha, gyokuro, or catechin.

Matcha: Five products containing *kabusecha* (*tencha*).

Instant products: Four products containing dextrin, ascorbic acid, and dried extracts with matcha.

2.3 Preparation of the Test Solution

Approximately 0.2 g of each green tea powder was weighed into a 2 mL microtube, followed by the addition of 1.5 mL of acetone. Extraction was performed via ultrasonication (Model AS52GTU, 40 kHz, Matsuura Co., Ltd.) for 10 min. The extract was then centrifuged (10 seconds, 1310–2960, Model MA1, Tomy Seiko Co., Ltd.), and the resulting supernatant was used as the test solution.

2.4 Circular Paper Chromatography (CPC) and Apparatus

CPC facilitates a wide separation zone due to omnidirectional diffusion, which is advantageous for obtaining the concentrations required for spectroscopic analysis.

1) Developing Bag: Fig. 1 illustrates a simplified developing apparatus that does not require saturation with the developing solvent.

A commercially available thick polyethylene zip-lock bag (140 × 160 mm, 0.06–0.08 mm thickness) was utilized. A cup stand (e.g., CF cup, polypropylene, approx. 7 × 85 mm, Chuo Kagaku Co., Ltd.) was placed inside the bag to support the circular filter paper during development.

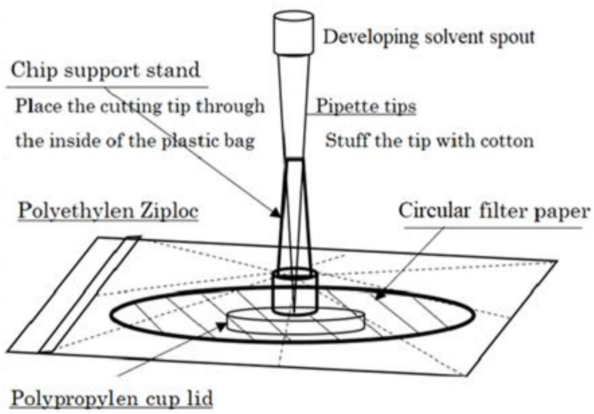

Fig. 1 Preparation of a developing bag for circular paper chromatography (CPC)

2) Developing solvent injector: The tip of a 1 mL pipette was packed with absorbent cotton to a depth of approximately 1 cm to adjust the flow rate (0.1–0.15 mL/min). The injector was inserted into the mobile phase inlet and positioned against the center of the filter paper, allowing the developing solvent to be gradually injected.

3) CPC conditions: The developing solvent (mobile phase) was prepared using a volumetric ratio of n-hexane, acetone, and 2-propanol (85:15:1, v/v/v; analytical grade, Kanto Chemical Co., Inc.) [12]. The flow rate was maintained at 0.1–0.15 mL/min.

4) Circular qualitative filter paper (No. 131, 125 mm, Advantec Toyo): Using a capillary tube, 0.1–0.5 mL of the CPC test solution was applied to the center of the filter paper (spot diameter: approx. 1.2 mm). Repeated spotting and drying were performed to concentrate the sample.

(5) Visible spectrophotometric analysis of Chl-a and Pheo-a

The developed CPC filter paper is presented in Fig. 2a) and Fig. 2b). For analysis and preservation, the filter paper was cut in half. The separation zones corresponding to ⑤ Chl-a (blue-green, *Rf* value: 0.62) and ② Pheo-a (gray, *Rf* value: 0.85), as shown in Fig. 1, were excised and transferred to 2 mL microtubes. Elution was performed by adding 1.5 mL of acetone to each tube to prepare the measurement solutions. These solutions were transferred to 1 mL disposable cuvettes (10 mm optical path length, Tokyo Glass Instruments), and their absorbance was measured across the visible wavelength range using a spectrophotometer (UV-1700, Shimadzu Corporation). The absorption maxima (λ_{max}) of the two species—662 nm for Chl-a and 666 nm for Pheo-a —were utilized as the measurement wavelengths. Based on the absorbance of the two compounds separated by CPC, the conversion ratio of Chl-a to Pheo-a was calculated [13].

2.1 Experiment on the Conversion of Chl-a to Pheo-a during Boiling of Fresh Tea Leaves

(1) Preparation of Heated Samples

Fresh tea leaves were weighed into 10 g portions, and four sets of seven samples each were prepared. The samples were immersed in a boiling water bath for 0, 20, 40, 60, 80, 100, and 120 seconds, respectively, followed by immediate cooling in ice water. After cooling, the leaves were wiped dry with paper towels to obtain the heated samples for analysis.

(2) Preparation of Chl-a and Pheo-a Test Solutions

A 5 g portion of each heated sample was mixed with 45 mL of ethanol and homogenized. The resulting homogenate was subjected to ultrasonic extraction (40 kHz, 10 min) and then centrifuged at 3000 × g for 10 min (CF12RX/9RX, Hitachi, Ltd.).

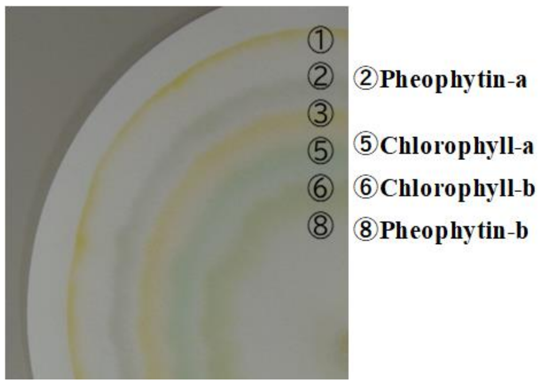

Fig. 2 a) CPC of chlorophyll-related compounds.

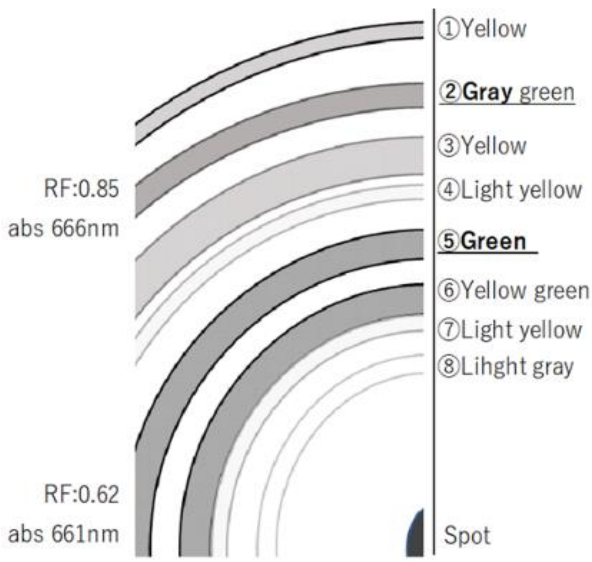

Fig. 2 b) CPC of chlorophyll-related compounds in green tea leave.

Chlorophyll-related compounds: ②Pheophytin-a (Rf 0.85), ⑤Chlorophyll-a (Rf 0.64), ⑥Chlorophyll-b, ⑧Pheophytin-b

The ethanol in the supernatant was removed via evaporation under reduced pressure. The residue was then eluted with diethyl ether (analytical grade, Kanto Chemical Co., Inc.). A small amount of anhydrous sodium sulfate (analytical grade, Kanto Chemical Co., Inc.) was added to the eluate for dehydration, yielding the final test solution. CPC and absorbance measurements were performed according to the procedures described in Section 2.

2.2 Conversion Test (Pheo-a/Chl-a) Related to Discoloration of Commercially Available Powdered Green Tea

The CPC separation and absorbance measurements for commercial powdered green tea samples were conducted in accordance with the protocols specified in Section 2.

3. Results and Discussion

(1) CPC of Chl-a and Pheo-a

As shown in Fig. 2a) and Fig. 2b), CPC analysis using circular filter paper and a development bag resulted in excellent separation of Chl-a and Pheo-a.

CPC analysis yielded long separation bands, allowing for the isolated material to be isolated at concentrations sufficient for spectroscopic analysis.

This method is not only simple to operate, but also allows for rapid and efficient analysis, with a development time of approximately 15 minutes and a development solvent volume of approximately 1.5 ml.

The CPC separation bands of Chl-a and Pheo-a were dissolved in acetone, and the visible absorption spectrum yielded absorbance wavelengths of 662 nm and 666 nm. The results are shown in Fig. 3.

(2) Changes in Pheos and Chls over time during the boiling process of fresh tea leaves

From the harvesting stage of fresh tea leaves to the thermal processing stage, Chls (-a, -b) converts to Pheos (-a, -b), causing discoloration. As a model, we investigated the changes over time during boiling, and the results are shown in Figs. 4 and 5.

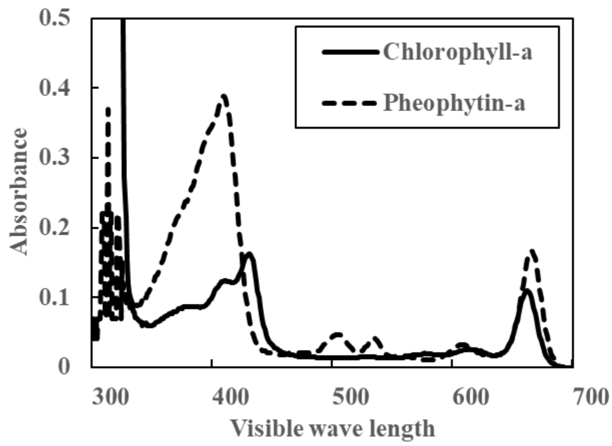

Fig. 3 Visible absorption spectra of chlorophyll-a and pheophytin-a separated by CPC.

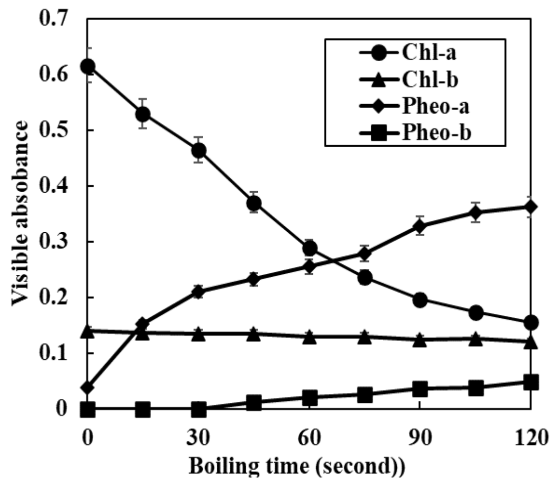

Fig. 4 Time course of Chl to Pheo in fresh tea leaves during boiling.

Chl: chlorophyll (-a, -b), Pheo: pheophytin (-a, -b)

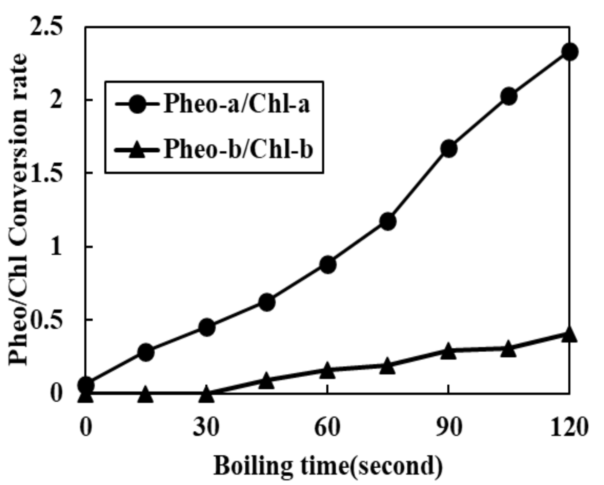

Fig. 5 Change in Pheo/Chl rate over time during boiling of fresh tea leaves (n = 3).

Chl: chlorophyll (-a, -b), Pheo: pheophytin (-a, -b)

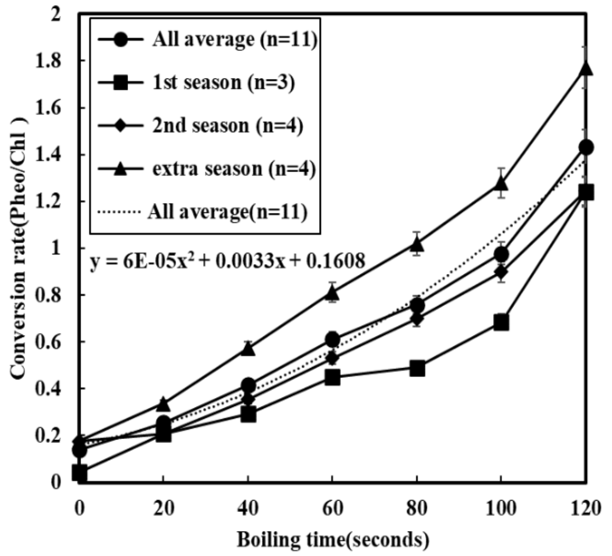

Fig. 6 Changes in the (Pheo-a / Chl-a) rate of fresh tea leaves in boiling water overtime at different harvest times.
Pheo-a: pheophytin-a, Ch-al: chlorophyll-a, (Pheo-a/Chl-a) rate: absorbance ratio

As boiling time progressed, Chls (-a, -b) decreased and Pheos (-a, -b) increased, showing an inversely proportional relationship. At the same time, the conversion rate of Pheo-a/Chl-a increased significantly. The conversion rate of Chl-b to Pheo-b (Pheo-b/Chl-b) was small and stable.

Based on these results, we focused on the unstable Pheo-a and Chl-a for analysis.

(3) Absorbance and Conversion Rate of Chl-a and Pheo-a during Boiling of Fresh Tea Leaves by Harvest Season

The first, second, and subsequent (autumn and winter) fresh tea leaves all showed an increased gray-green color and discoloration during boiling. The change in the conversion rate (Pheo-a/Chl-a) is shown in Fig. 6.

The results showed that the ease of conversion was highest in subsequent tea leaves (autumn and winter), followed by second and first, indicating a trend depending on the harvest season.

Contributing factors to this include meteorological conditions such as sunlight and temperature on the tea plants, fertilization conditions, and tea harvesting and transportation methods [1].

The conversion rate (Pheo/Chl) curve during boiling was considered useful as an indicator of the degree of heating and discoloration of tea, including green tea, and ultimately the brewing quality and flavor of tea.

(4) Conversion rate (Pheo-a/Chl-a) of commercially available powdered green teas

The conversion ratios for the powdered green teas shown in Table 1 were: instant green tea (0.86), powdered green tea (1.21), and Matcha (0.38).

The secondary ingredients used in these products were Matcha, dextrin, and ascorbic acid (4/4 cases) for instant tea and powdered green tea (1/6 cases). Catechin-containing powdered green tea (1/6 cases) was used. Matcha was the only ingredient (5/5 cases).

Table 1 Absorbance of Pheo-a and Chl-a related to discoloration of powdered green tea and their conversion rates (Pheo-a/Chl-a).

ABS	Chl-a	Pheo-a	Pheo-a/Chl-a
Instant tea	0.30	0.26	0.88
	0.17	0.16	0.95
	0.05	0.05	0.96
	0.07	0.04	0.67
[Av]	0.15	0.13	0.86
Powdered green tea	0.26	0.53	2.02
	0.30	0.22	0.74
	0.30	0.57	1.89
	0.15	0.23	1.54
	0.46	0.29	0.62
	0.34	0.15	0.45
[Av]	0.30	0.33	1.21
Old	0.00	0.11	0.00
Matcha	0.45	0.15	0.34
	0.47	0.28	0.60
	0.42	0.21	0.49
	0.53	0.14	0.26
	0.42	0.10	0.23
[Av]	0.46	0.18	0.38
Fresh tea leaves(first harvest)	0.51	0.01	0.02
	0.55	0.03	0.06
	0.48	0.02	0.05
[Av]	0.51	0.02	0.04

Instant tea: Dried tea after extraction (See List2(2)b above)
Powder: Powdered green tea
Old: Old powdered green tea, F. leaves: Flesh tea leaves

Highly processed instant teas showed low conversion rates. This suggests the influence of Matcha and additives, and it was necessary to distinguish and evaluate the conversion rate based on the label.

Tea leaves are picked taking into consideration the climate, harvest time and varietal characteristics, transported at low temperatures, processed (steamed, beaten, rolled, dried), finished (roasted, blended) and then branded. The Pheo-α/Chl-α conversion rate of green tea is expected to be an indicator of brand preparation [7].

4. Conclusions

(1) Simple analysis of Chl-a and Pheo-a components in powdered green tea

Analysis of Chl-a and Pheo-a components, which are involved in the discoloration of the appearance of green tea, was investigated. The CPC using the designed simple spreading bag effectively separated Chl-a and Pheo-a components. The absorbance of the separated Chl-a and Pheo-a was measured, and the conversion rate (Pheo-a/Chl-a) was calculated and evaluated as the degree of discoloration.

(2) Boiling experiment to convert Chls (-a, -b, green) into fresh tea leaves to Pheos (-a, -b, gray)

1) Chl-a was readily converted to Pheo-a by boiling, but Chl-b was stable and its conversion rate to Pheo-b was low. Therefore, the conversion rate from Chl-a to Pheo-a was used as an indicator of discoloration.

2) Harvested fresh tea leaves gradually discolored to gray-green with increasing boiling time.

The order of susceptibility to discoloration was the additional, second, and first harvest periods. This was thought to be influenced by the weather and number of harvests at harvest time.

3) Green tea with a higher conversion rate (Pheo-a/Chl-a) indicated a stronger heat treatment. In other words, the conversion rate is related to the appearance and flavor of tea leaves and serves as an indicator of the degree of heat treatment during the manufacturing process.

(3) Conversion rate (Pheo-a/Chl-a) of commercially available powdered green tea

The average Pheo-a/Chl-a conversion rate of commercially available powdered green tea was lowest for matcha, followed by instant green tea and powdered green tea. Powdered green tea has a wide range of Pheo-a/Chl-a and contains secondary ingredients and additives, so it is necessary to separate it based on the label and evaluate the conversion rate, etc.

(4) Heat Treatment and Color Indicators and Conversion Rate (Pheo-a/Chl-a)

This conversion rate evaluates the deterioration of green tea caused by transportation of flesh tea leaf after harvesting, heating manufacturing process (steaming, softening, drying/rough tea processing, and finishing), storage, etc. It was thought that this would be useful for achieving and maintaining the specified quality.

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