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Improving the antibacterial activity against *Staphylococcus aureus* of composite sheets containing wasted tea leaves by roasting

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Abstract We used various kinds of wasted tea leaves to develop composite sheets with antibacterial properties. Antibacterial tests showed that the number of viable bacterial cells for the sheet containing wasted green tea leaves was around 10^6–10^7 CFU/ml after 18 h culture compared to 10^8 CFU/ml for a tea-free sheet. This indicates that cell growth was significantly inhibited. For sheets containing other types of tea leaves (oolong, black, *hojicha*, and *pu-erh*), living cells were not found, indicating that these sheets had superior antibacterial effects. Living cells were also not found in sheets containing wasted black tea leaves or roasted tea leaves at a concentration of 60% by weight after 6 h cultivation. Therefore, roasting treatment of wasted green tea leaves was examined to improve the antibacterial activity of the sheet. In particular, the focus was on structural conversion of catechins by heating.

Key words Recycling · Wasted tea leaves · Composite sheet · Roasting · Antibacterial activity

Introduction

Tea is well known for many reasons, including for its antibacterial and health-related properties.[1–3] The antibacterial properties of tea provided benefits to society even before the advent of science; old traditions such as "takeout lunch with tea" and "sushi with tea" were driven by empirical knowledge that drinking tea is effective for relieving the symptoms of bacterial diarrhea.[4] Recently, consumption of tea leaves has increased drastically because of the ready availability of teas in cans and plastic bottles. Presently, large amounts of wasted tea leaves, which still contain catechins and other useful chemicals, are being dumped as industrial wasted. Therefore, we have attempted to produce sheet containing wasted tea leaves as an effective utilization of this wasted product.[5,6] In a previous study, *Staphylococcus aureus*, a bacterium that causes food poisoning, was used to examine the antibacterial properties of sheet containing wasted tea leaves from green tea and black tea. The results suggested that sheet containing used black tea leaves possessed excellent antibacterial properties.[5,6] Our current study attempts to improve the antibacterial properties of sheet containing wasted green tea leaves by roasting the leaves before preparing the sheet, which is then examined for the effects of this treatment on its antibacterial properties.

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Materials and methods

Preparation of wasted tea leaves

The study involved the following five kinds of tea leaves: green tea (*sencha*), oolong tea, black tea, *pu-erh* tea, and *hojicha*. These tea leaves are fermented to different levels by different methods. Green tea leaves were obtained from Ito En, oolong tea leaves from Nihoncha Hanbai, black tea leaves from Mitsui Norin, *hojicha* leaves from Nihoncha Hanbai, and *pu-erh* tea leaves from Tenjin Seicha.

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, the temperature was set to 90°C for extraction. For *hojicha*, 300 g of tea leaves was added to 3.0 l of distilled water, and the mixture was heated to 90°C for 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 extraction.[5–9]

Roasting method

Tea leaves and wasted tea leaves, in 100 g quantities, were placed in a frying pan 135 mm in diameter and 70 mm in depth on a hot plate and stirred with a stainless steel spoon for roasting at desired temperatures. The wasted tea leaves had previously been air-dried for 24 h to completely evaporate moisture before the roasting treatment. Roasting conditions were selected using as reference information common roasting conditions of 10 min at about 180°C.

Preparation of sheets containing wasted tea leaves

Sheets containing wasted tea leaves were prepared by the method reported previously.[5–9] 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 wasted tea leaf content was adjusted to 0 wt%, 20 wt%, or 60 wt% in the formulation; the latex binder content was 0.3 wt% to the combined amount of the wasted tea leaves and the pulp in all the formulations. The pulp used was prepared by refining Canadian softwood bleached kraft pulp to a Canadian standard freeness of 550 ml with a laboratory Niagara-beater (Toyo Tester Kogyo). A latex binder (Aica Aibon RAX117 from Aica Kogyo) made of styrene-butadiene rubber (SBR) was used to improve the adhesion between pulp and wasted tea leaves. The binder is mainly used for sheet processing, which commonly involves strengthening and coating of sheets. 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 was used to adjust the slurries to a sheet weight of 100 mg/m^2 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 sheets containing wasted tea leaves for irradiation treatment as follows.

Tests of antibacterial properties

Staphylococcus aureus (NBRC 12732) used for the antibacterial evaluation was obtained from the Incorporated Administrative Agency, National Institute of Technology and Evaluation. The bacterium is a gram-negative coccus and is known as a source of purulent diseases as well as a pathogen of food poisoning. JIS-L-1902 was used as the reference for testing antibacterial properties. A sample of 0.20 g from each of the tea leaves, wasted tea leaves, and composite sheets containing the wasted tea leaves was transferred into a vial; in the case of tea drinks, the volume of the samples was 0.20 ml. The vials with the samples were autoclaved in a BS-245 autoclave from Tomy Kogyo at 121°C for 15 min. Peptone (1.0 wt%) and yeast extract (0.5 wt%) from Becton Dickinson Company and sodium chloride (0.5 wt%) were used to prepare peptone water of a prescribed concentration. The peptone water was used to prepare a *Staphylococcus aureus* (NBRC 12732) suspension at a concentration of $1.0 \pm 0.3 \times 10^5$ CFU/ml. Each autoclaved sample was inoculated with 0.10 ml of the suspension, tightly sealed, and incubated at 37° ± 1°C for 18 h.

To each of the incubated vials, 10 ml of a rinsing physiological saline, which was adjusted to a prescribed concentration with sodium chloride (0.85 wt%) and Tween 80 (0.20 wt%) from Sigma Chemicals, was added, and the vials were shaken to disperse the bacterial cells. Physiological saline prepared with sodium chloride (0.85 wt%) was added to the stock dispersion of each sample in order to dilute them to the desired concentrations of up to 10^7-fold. A mannitol salt medium (Ganule) from Nissui Pharmaceutical, adjusted to 11.1 wt%, was inoculated with each of the diluted bacterial suspensions. The adopted inoculation method[10] involved dropping 5 μl of the diluted suspension at five spots in each of the four sections of the medium as described previously.[5,6] The Petri dishes were placed inverted in an incubator at 37° ± 1°C for 44 h. Grown colonies were counted and multiplied by the dilution ratios to calculate the numbers of viable cells.

In order to investigate the effect of irradiation alone, the sheet was shielded from light with aluminum foil, left in an irradiation chamber for 20 h, and tested for antibacterial activity.

The parameters evaluated were log C (the common logarithm of the number of viable cells), the bacteriostatic activity (= log N_2 – log N_3), and bactericidal activity (= log N_1 – log N_3), where N_1 is the initial cell number, N_2 is the viable cell number dropped on rayon fiber after 18 h of incubation, and N_3 is the viable cell number dropped on rayon fiber containing complex after 18 h of incubation.

Color hue evaluation

Samples were placed on a standard white plate of barium sulfate and measured for reflectance at 300–700 nm with an ultraviolet spectrophotometer (UV-3100, Shimadzu, Japan). The slit width and the sampling pitch were set at 2.0 nm and 0.5 nm, respectively. Reflectance values were corrected against 100% using the reflectance of the standard white plate.

Spectroscopic analysis using Fourier transform infrared (FT-IR) spectroscopy

An infrared spectrophotometer (FT-720, Horiba, Japan) equipped with a horizontal attenuated total reflectance (ATR) device was employed to obtain infrared absorption spectra of the sheets containing wasted tea leaves. The measurement conditions for all samples were as follows: 4-cm^{-1} resolution, 1000 scans, and a triglycine sulfate crystal (TGS) detector.

Results and discussion

Antibacterial properties of tea leaves with different degrees of fermentation, tea drinks, and wasted tea leaves

Because leaves of green tea, oolong tea, black tea, and *pu-erh* tea are fermented to differing degrees with oxidase, they are likely to possess differing levels of antibacterial activity. Further, *hojicha*, a green tea prepared by roasting *sencha* (green tea) or *bancha* (coarse green tea) leaves from the second or third flush of the season, is predicted to differ in antibacterial activity from green tea without any treatment. For our project, these different kinds of tea leaves were brewed to produce tea drinks and wasted tea leaves, after which *S. aureus* was used to perform an antibacterial evaluation of the five kinds of tea leaves.

Table 1 shows the results of the antibacterial evaluation of tea leaves, tea drinks, and wasted tea leaves. The number of viable cells in the normal cultivation medium increased from 1.0×10^5 CFU/ml before incubation to 1.49×10^{10} CFU/ml after 18 h of incubation with the bacterium alone. Thus, the number of *S. aureus* cells increased 10^5-fold. In contrast, no viable cells were found in the presence of the five kinds of tea leaves, demonstrating that tea leaves possessed strong antibacterial properties. Catechins and theaflavins have previously been identified as chemical components of tea leaves known to possess antibacterial properties against food-poisoning bacteria such as *S. aureus*.[4] The present experiment confirmed that all of the tea leaves had sufficient levels of antibacterial properties.

The five tea drinks from the tea leaves were also evaluated for antibacterial properties against *S. aureus*, as shown in the same table. The green tea drink showed 4.00×10^3 CFU/ml after 18 h of incubation, whereas oolong tea, black tea, *hojicha*, and *pu-erh* tea drinks showed excellent antibacterial properties with no viable cells found. This comparison of the five tea drinks clarified that the green tea drink was inferior to the other tea drinks in antibacterial properties. However, when compared with the incubation of the bacterial suspension alone (1.49×10^{10} CFU/ml), the green tea drink clearly inhibited bacterial multiplication (4.00×10^3 CFU/ml). Therefore, it was shown that the tea drinks as well as the tea leaves possess antibacterial activity. This is assumed to result from the extraction of catechins and theaflavins by hot water during the preparation of tea drinks.

The wasted tea leaves remaining after brewing were similarly tested, with results also shown in Table 1. None of the five kinds of wasted tea leaves (green tea, oolong tea, black tea, *hojicha*, and *pu-erh* tea) showed viable cells, demonstrating excellent antibacterial properties. This result suggests that sufficient amounts of antibacterial chemical components remained in the wasted tea leaves even after brewing. This fact supports the possibility of reusing the wasted tea leaves as antibacterial materials.

Antibacterial properties of sheets containing wasted tea leaves

As described above, all five kinds of wasted tea leaves (green, oolong, black, *hojicha*, and *pu-erh*) possessed excellent antibacterial properties. Thus, they were employed for the preparation of sheets with antibacterial activity. As a control, a piece of sheet made of 100% by weight (wt%) pulp containing no wasted tea leaf was also evaluated. Table 2 shows the results of evaluating sheets containing 20 wt% and 60 wt% tea leaves.

The number of viable cells for the inoculated 100 wt% pulp sheet increased from 1.0×10^5 CFU/ml before incubation to 1.64×10^8 CFU/ml after 18 h of incubation. In contrast, the number of viable cells after incubation increased to 1.39×10^7 CFU/ml and 5.36×10^6 CFU/ml, respectively, for sheets containing wasted green tea leaves at 20 wt% and 60 wt%. Sheets containing wasted oolong tea leaves exhibited viable cell counts of 1.60×10^4 CFU/ml and zero for 20 wt% and 60 wt% wasted tea leaf content, respectively. For sheets containing wasted leaves from black, *hojicha*, and *pu-erh* teas, no viable cells were found for either 20 wt% or 60 wt% wasted tea leaf content. When compared with the other types of composite sheets, sheets containing wasted green tea leaves showed a poor antibacterial effect. Even the sheet containing the higher level of wasted green tea leaves did not exhibit a marked inhibition of bacterial multiplication, although it did show more antibacterial activity than did the 100 wt% pulp sheet (Fig. 1).

Another antibacterial evaluation was performed altering the incubation time after inoculation. It was expected that incubation times shorter than 18 h could clarify the differ-

Table 1. Antibacterial properties of different kinds of tea against *Staphylococcus aureus*

Kinds of tea	Incubation time (h)	Tea leaves		Tea		Wasted tea leaves	
		Viable bacteria (CFU/ml)	Log C[a]	Viable bacteria (CFU/ml)	Log C	Viable bacteria (CFU/ml)	Log C
Initial	0			1.0×10^5	5		
Green tea	18	ND[b]	–	4.00×10^3	3.60	ND	–
Oolong tea	18	ND	–	ND	–	ND	–
Black tea	18	ND	–	ND	–	ND	–
Hojicha	18	ND	–	ND	–	ND	–
Pu-erh tea	18	ND	–	ND	–	ND	–
Bacteria only	18			1.49×10^{10}	10.17		

[a] Viable bacteria number
[b] Not detected (<440)

Table 2. Antibacterial properties of sheets containing wasted tea leaves against *Staphylococcus aureus*

Sample	Kinds of tea	Wasted tea leaves content (wt%)	Incubation time (h)	Antibacterial properties			
				Viable bacteria (CFU/ml)	Log C^a	Bacteriostatic activity	Bactericidal activity
Initial	–	–	0	1.0×10^5	5	–	–
Sheets containing wasted tea leaves	Green tea	20	18	1.39×10^7	7.14	1.08	–2.14
		60	18	5.36×10^6	6.73	1.49	–1.73
	Oolong tea	20	18	1.60×10^4	4.20	5.97	0.8
		60	18	ND[b]	–	–	–
	Black tea	20	18	ND	–	–	–
		60	18	ND	–	–	–
	Hojicha	20	18	ND	–	–	–
		60	18	ND	–	–	–
	Pu-erh tea	20	18	ND	–	–	–
		60	18	ND	–	–	–
Pulp sheet	–	0	18	1.64×10^8	8.22	–	–

[a]Viable bacteria number
[b]Not detected (<440)

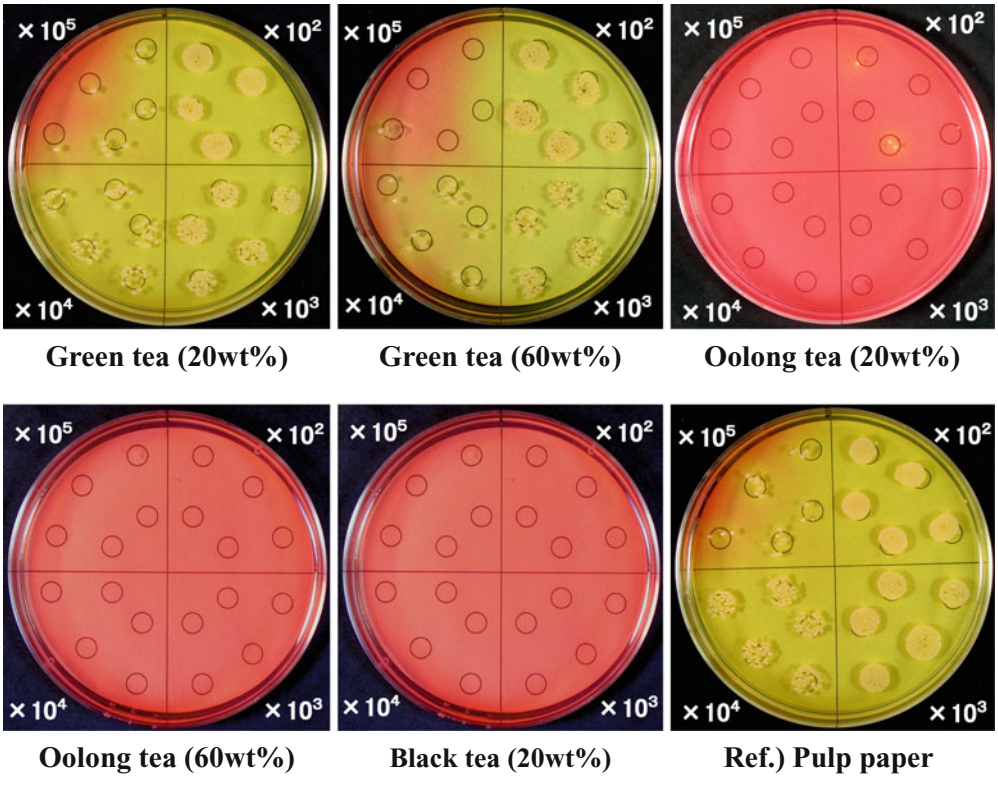

Fig. 1. Antibacterial properties of sheets containing wasted tea leaves against *Staphylococcus aureus*

ences in antibacterial activity among the sheets. The samples tested were sheets containing wasted tea leaves at 60 wt% and a control of 100 wt% pulp sheet. Incubation times were altered over the range 1–18 h.

The results of these tests are shown in Table 3. All the sheets inoculated with the bacterial suspension adjusted to 1.0×10^5 CFU/ml were found to have viable cells at 10^4–10^5 CFU/ml after 1 h incubation. These numbers were not significantly different from the time of inoculation, indicating no bacterial multiplication. After 3 h incubation, the number of viable cells decreased to about 10^4 CFU/ml in the sheets containing wasted black or *hojicha* tea leaves. None of the sheets containing wasted leaves from the other teas (green, oolong, and *pu-erh*) showed a significant change in the number of viable cells from the initial number at inoculation. After 6 h of incubation, sheets containing wasted tea leaves from black tea and *hojicha* tea no longer contained viable cells. In contrast, sheets containing wasted oolong or *pu-erh* tea leaves retained viable cells for up to 9 h of incubation but showed none after 18 h. For the sheet containing the wasted green tea leaves, the number of viable cells reached 9.20×10^6 CFU/ml after 18 h incubation.

From the above results, the sheets containing wasted tea leaves from black tea and *hojicha* tea were found to be superior in antibacterial properties among the five composite sheets examined. The antibacterial ability of the other

Table 3. Viable bacteria (CFU/ml) on sheets containing wasted tea leaves after various incubation times

Sample	Kinds of tea	Wasted tea leaves content (wt%)	Incubation time (h)					
			0	1	3	6	9	18
Sheets containing wasted tea leaves	Green tea	60	1.0×10^5	9.20×10^4	1.28×10^5	1.44×10^5	4.21×10^5	9.20×10^6
	Oolong tea	60	1.0×10^5	4.80×10^4	7.60×10^4	1.20×10^4	4.00×10^3	ND[a]
	Black tea	60	1.0×10^5	5.60×10^4	8.00×10^3	ND	ND	ND
	Hojicha	60	1.0×10^5	6.89×10^4	9.41×10^3	ND	ND	ND
	Pu-erh tea	60	1.0×10^5	1.48×10^5	1.24×10^5	4.80×10^4	8.40×10^4	ND
Pulp sheet	–	0	1.0×10^5	52.0×10^4	2.52×10^5	5.16×10^5	2.76×10^7	1.68×10^8

[a] Not detected (<440)

sheets can be ranked in descending order as follows: oolong, *pu-erh*, and green. Green tea is rich in catechins, which are more water soluble than theaflavins with which black tea and *hojicha* abound. It was then inferred that catechins dissolved in water ran off easily in the sheet-making process using wasted green tea leaves and did not remain in the sheet containing wasted green tea leaves. This catechin runoff is estimated to be the likely cause for the inferior antibacterial property of the sheet compared to sheets containing other wasted tea leaves.

Green tea is the most consumed tea in Japan. It is thus disappointing that the sheet containing wasted green tea leaves is inferior in antibacterial property to sheets containing other wasted tea leaves. It is essential to improve the antibacterial property of wasted green tea leaves for the recycling of wasted tea leaves.

Improvement of antibacterial properties of composite sheets containing wasted tea leaves by roasting

It is necessary to remove the influence of growth conditions and harvested parts of tea leaves in order to correctly evaluate the effects of roasting treatment to improve their antibacterial properties. Green tea leaves were roasted under various conditions, and the roasted tea leaves were brewed to obtain the wasted green tea leaves that were then ground and mixed with pulp to prepare sheets.

Sheets containing 60 wt% wasted tea leaves were evaluated for antibacterial properties. The sheet made with wasted tea leaves treated at 200°C or higher for 15 min or longer was found to have good antibacterial properties. From this result, it can be stated that roasting is the likely reason for the strong antibacterial properties of the sheets containing wasted *hojicha* leaves. Heat from roasting is assumed to allow catechins to be converted into theaflavins, resulting in increased antibacterial activity.

Wasted tea leaves from *hojicha* make up only a small part of the wasted tea leaves discarded as industrial wasted, whereas wasted *sencha* and *bancha* tea leaves as nonroasted green teas make up the major portion. Table 1 clearly shows that wasted green tea leaves still exhibit antibacterial properties; thus, sufficient amounts of catechins probably remain in the wasted tea leaves. Therefore, we attempted to use a roasting treatment on wasted green tea leaves to increase their antibacterial properties.

To study the effect of roasting temperature, used green tea leaves were roasted for 15 min at temperatures ranging from 180° to 220°C. The roasted leaves were ground and mixed with pulp to prepare sheets. As shown in Fig. 2, the prepared sheets show that intensity of the greenish color hue in the sheets decreased and intensity of blackish color increased as the roasting temperature got higher.

The sheets were also tested for antibacterial properties using *S. aureus*; sheets containing nonroasted green tea and the 100 wt% pulp were used as controls. It was clear that roasting for 15 min decreased the number of viable cells, indicating improvement of antibacterial properties of the sheets. It should be noted that no viable cells were recognized when the roasting temperature reached 200°C or higher, whereas the number of viable cells was 3.28×10^5 CFU/ml at a roasting temperature of 190°C. This implies that 200°C is a critical temperature for roasting to obtain antibacterial properties.

To examine the effect of roasting time, roasting temperature was fixed at 200°C, and roasting time was altered over a range of 8 to 20 min. The roasted wasted green tea leaves were ground and mixed with pulp to prepare sheets. Figure 3 shows the prepared sheets containing wasted green tea leaves, and Fig. 4 shows the reflectance measurements of the sheets at 300–700 nm obtained with a spectrophotometer. A shift in color hue due to the roasting was seen, with blackish color hue intensity increasing as roasting time extended from 10 to 12 min. Sheets were also found to decrease in reflectance substantially, particularly in the visible light region near 500–600 nm. In addition, roasting for 12 min or longer apparently increased the blackish color hue intensity of the sheets (Fig. 3). In fact, a comparison between Fig. 2 and Fig. 3 indicates that increasing the roasting temperatures exerted greater effects on color hue change than did longer roasting times.

Sheets containing wasted green tea leaves that had been roasted for various times were also examined for antibacterial properties (Table 4). Sheets containing wasted green tea leaves roasted for short periods (ranging from 8 to 10 min) had relatively small numbers of viable cells (in the range of 10^5–10^6 CFU/ml). This bacterial concentration value is nearly equal to that of the inoculum (1.0×10^5 CFU/ml) and clearly lower than that (5.19×10^7 CFU/ml) found in the sheet with nonroasted wasted green tea leaves. Moreover, roasting for 12 min or longer resulted in no detection of

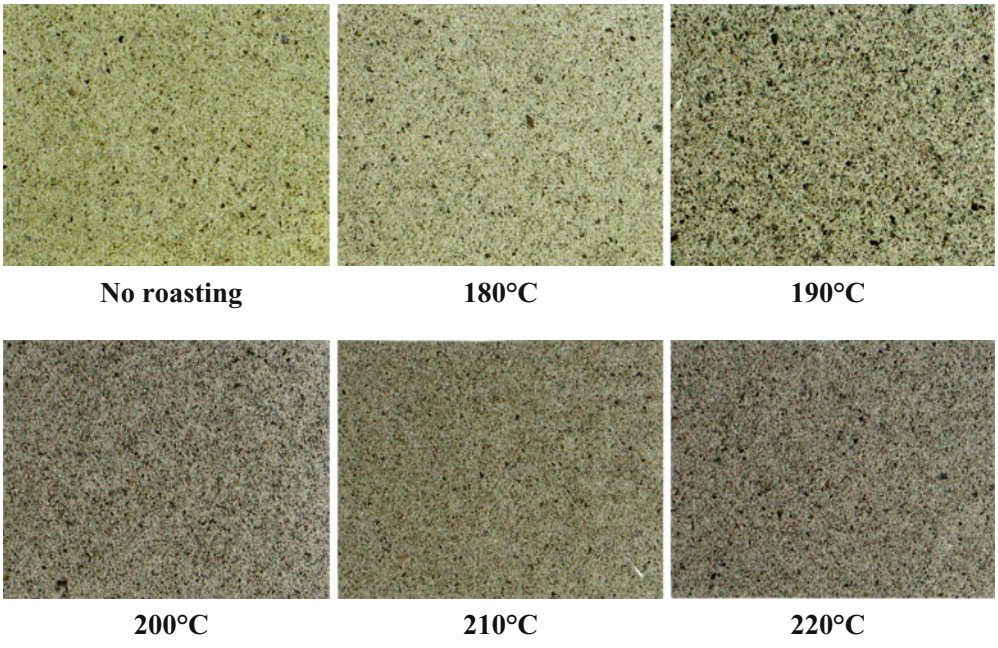

Fig. 2. Sheets containing wasted green tea leaves roasted at different temperatures for 15 min (sample size: 4 cm × 5 cm)

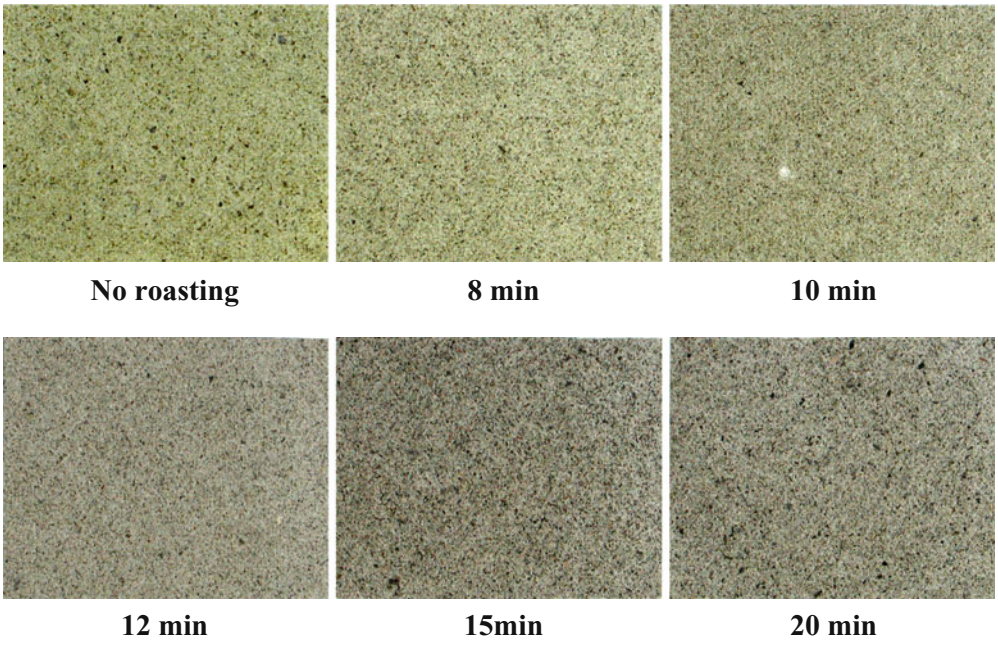

Fig. 3. Sheets containing wasted green tea leaves roasted for different lengths of time at 200°C (sample size: 4 cm × 5 cm)

viable cells. These results show that roasting at 200°C facilitated oxidation more than roasting at 180°C, and further, that roasting for 12 min or longer was necessary to obtain excellent antibacterial properties. Thus, improving the antibacterial properties of sheet containing wasted green tea leaves by roasting the wasted leaves may be regarded as a promising means for effectively recycling wasted green tea leaves, which are now discarded as industrial wasted. Volatile substances given off from roasting tea leaves have not been studied. This is an important research subject for the future.

Characterization using infrared spectroscopy of the formation of theaflavins by roasting

Catechins, which possess antibacterial activity, are known to decrease in amount as the degree of tea leaf fermentation proceeds from green tea through oolong tea to black tea.[11] *Pu-erh* tea is known to contain extremely low levels of catechins.[11] The antibacterial components in tea leaves are not only catechins but also theaflavins, which are derived from catechins through oxidation and polymerization and possess higher antibacterial activities than catechins do.[4]

Table 4. Antibacterial properties of sheets containing wasted green tea leaves roasted under different conditions

Sample	Wasted tea leaves content (wt%)	Roasting conditions		Incubation time (h)	Antibacterial properties			
		Temp. (°C)	Time (min)		Viable bacteria (CFU/ml)	Log C^a	Bacteriostatic activity	Bactericidal activity
Initial	–	–	–	0	1.0×10^5	5	–	–
	60	No roasting		18	5.19×10^7	7.72	0.71	–2.72
	60	180	15	18	2.36×10^7	7.37	1.06	–2.37
	60	190	15	18	3.28×10^5	5.51	2.92	–0.51
Sheets containing	60	200	15	18	ND[b]	–	–	–
wasted tea	60	210	15	18	ND	–	–	–
leaves	60	200	8	18	5.08×10^5	5.70	2.73	–0.70
	60	200	10	18	1.64×10^6	6.21	2.21	–1.21
	60	200	12	18	ND	–	–	–
	60	200	15	18	ND	–	–	–
Pulp sheet	0	–	–	18	2.72×10^8	8.43	–	–

[a] Viable bacteria number
[b] Not detected (<440)

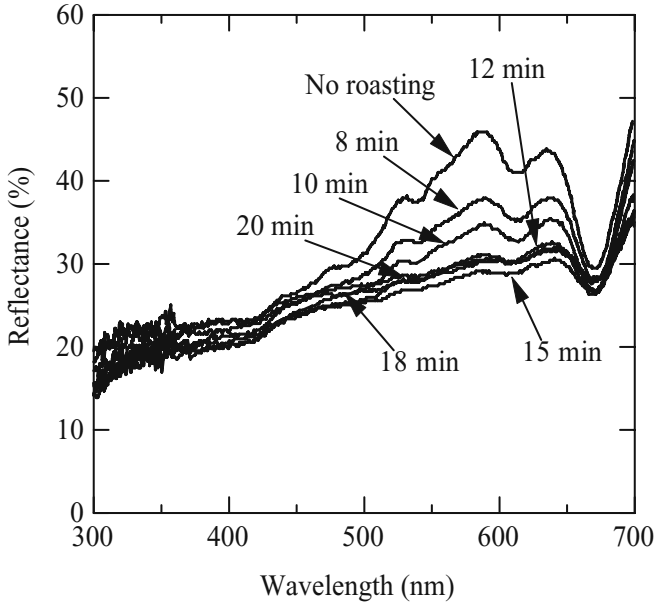

Fig. 4. Reflectance of sheets containing roasted wasted green tea leaves versus wavelength for different roasting times at a roasting temperature of 200°C

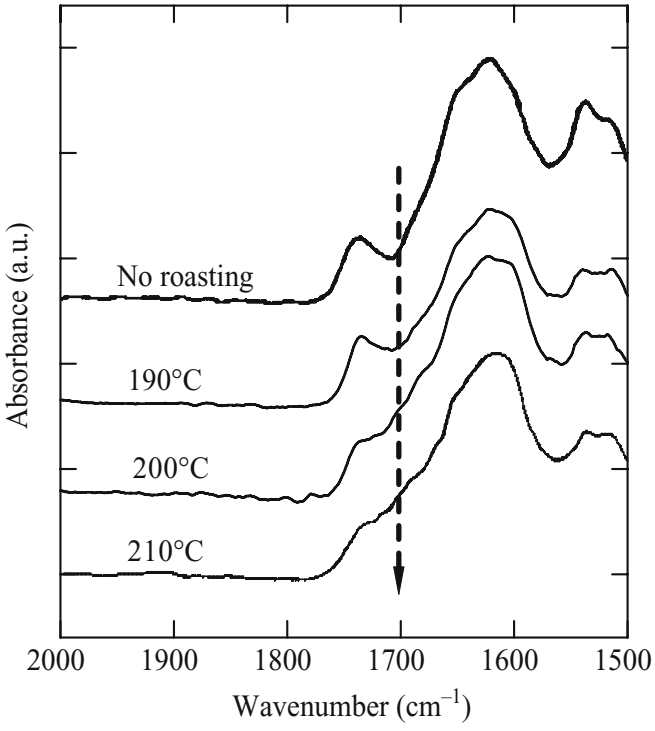

Fig. 5. Fourier transform infrared (FT-IR) spectra of sheets containing wasted green tea leaves roasted at different temperatures for 15 min. *Dotted line* shows absorption peak of a carbonyl group

It was necessary to analyze the components of the sheets containing wasted tea leaves prepared in this study. Figure 5 shows ATR-IR spectra of sheets containing 60 wt% wasted green tea leaves that had been roasted at various temperatures for a fixed time of 15 min. When wasted green tea leaves were not roasted, no absorption peak appeared near 1700 cm^{-1} at the roasting temperature of 190°C. In contrast, an absorption peak did appear near 1700 cm^{-1} at roasting temperatures of 200° or 210°C, and the spectrum showed that the depressions changed into shoulder shapes. This result corresponds to the antibacterial properties listed in Table 4. Sheets containing wasted green tea leaves roasted at 190°C, which showed no absorption peak near 1700 cm^{-1}, also did not exhibit significant antibacterial properties, whereas the sheet containing leaves roasted at 200°C showed an absorption peak near 1700 cm^{-1} and possessed excellent antibacterial properties.

Figure 6 presents the IR spectra of sheets containing wasted green tea leaves roasted at 200°C for varying times. Although the sheet containing nonroasted wasted green tea leaves showed no absorption peak near 1700 cm^{-1}, all the sheets roasted at 200°C showed an absorption peak near 1700 cm^{-1}. Moreover, when compared with the spectra of the sheets containing leaves roasted for 8 and 10 min, those of the sheets containing leaves roasted for 12 min or longer had larger absorption peaks.

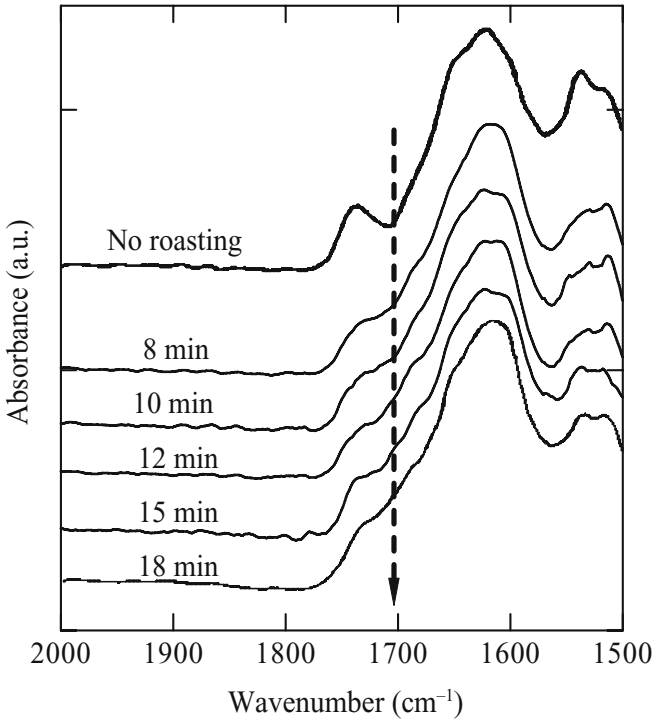

Fig. 6. FT-IR spectra of sheets containing wasted green tea leaves roasted for different lengths of time at 200°C. *Dotted line* shows absorption peak of a carbonyl group

It is possible that the roasting treatment oxidized and polymerized catechins in wasted tea leaves into theaflavins. The oxidation polymerization to theaflavins was inferred to be the cause for the sheet containing roasted wasted tea leaves to show an absorption peak near 1700 cm^{-1}. However, this theory is not supported by analytical data and is solely based on inference. This issue will remain a subject for future research.

Theaflavins, which are present in the roasted sheets and which have galloyl and pyrogallol groups, containing the hydroxyl group, likely have a strong influence on antibacterial action. During the tea drinking process, catechins that are plentiful in green tea leaves are readily extracted by hot water, but theaflavins are much less easily extracted. Catechins easily dissolve in water and run off in the sheet-making process[6], whereas theaflavins are inferred to be more likely to remain in sheets containing wasted tea leaves after the sheet-making process. The efficiency in the extraction of catechins was considered as a reasonable explanation for the difference in antibacterial properties between the sheets containing wasted tea leaves from green tea and from black tea. It is planned to perform analyses and report the results in the future.

Conclusions

Wasted *sencha* (green tea) leaves were roasted and used to prepare sheets, which were examined for antibacterial properties. The result revealed that roasting at 200°C for 15 min or longer markedly improved the antibacterial properties of the sheets. These sheets showing full antibacterial properties had a changed IR spectrum exhibiting an absorption peak near 1700 cm^{-1}. As described above, the sheets containing roasted wasted green tea leaves exhibited substantially improved antibacterial properties. Roasting is expected to be a promising means for recycling wasted green tea leaves currently discarded as industrial wasted.

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