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Changes in the Concentrations of Acrylamide, Selected Odorants, and Catechins Caused by Roasting of Green Tea

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This research aims to optimize roasted green tea (Houjicha) processing by using roasting treatments to achieve acrylamide mitigation without compromising the quality. 2-Ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine were identified as potent odorants by aroma extract dilution analysis. In preliminary sensory experiments, the desirable Houjicha flavor was produced in products roasted at 160 °C for 30 min and at 180 °C for 15 min. Under these conditions, potent odorants were formed at levels adequate for contributing to the Houjicha flavor. Acrylamide amounts in tea infusions were 2.0 and 4.0 μg/L by roasting at 160 °C for 30 min and at 180 °C for 15 min, respectively. Compared to roasting at 180 °C, the degradation of tea catechins was suppressed by roasting at 160 °C. Hence, roasting at 160 °C for is recommended for Houjicha processing for acrylamide mitigation, formation of potent odorants, and suppression of degradation of tea catechins.

KEYWORDS: Acrylamide; alkylpyrazine; catechin; Houjicha; aroma extract dilution analysis

INTRODUCTION

The discovery of acrylamide in heated foodstuffs has raised public concern (*1*) because the International Agency for Research on Cancer has classified this compound as "probably carcinogenic to humans" (*2*). In 2005, the Joint FAO/WHO Expert Committee on Food Additives recommended that appropriate efforts should be made to reduce the concentration of acrylamide in foodstuffs (*3*). It is well-known that the Maillard reaction plays a major role in acrylamide formation, and asparagine and carbonyl compounds (i.e., reducing sugars) are essential precursors of acrylamide (*4–6*). Many factors such as the initial reactant concentration of the precursors and their ratio and processing conditions (i.e., heating time, temperature, and moisture level in the raw material, pH, and the presence of additives) have been shown to influence the levels of acrylamide formed in heated foodstuffs. Moreover, the amount of acrylamide formed depends not only on the type of food but also on the storage conditions (i.e., temperature and duration) of the raw material as well as the cultivation conditions of the raw material (i.e., plant cultivar, soil, and harvesting time). As mentioned above, the key factors in reducing the acrylamide content in heated foodstuffs would be suppressing the Maillard reaction and preparing the raw material such that it has lower levels of acrylamide precursors. Reviews on acrylamide and its mitigation are available (*7–9*).

Roasted green tea (Houjicha) contains a considerable amount of acrylamide (approximately 250–1880 ng/g), and the level of asparagine in the tea material and the roasting conditions (roasting temperature and time) strongly influence the process of acrylamide formation (*10*). In the tea industry, Houjicha is processed from 170 to 200 °C for approximately 10 min. The desirable flavor of Houjicha is mainly roasty. The major constituents of volatile extracts from Houjicha are heterocyclic compounds, in particular, pyrazines (*11*). In general, pyrazines have roasty, nutty, earthy, burnt, and sweet odors with low threshold values. However, it remains unclear whether the potent odorants in Houjicha are pyrazines. Current investigations using aroma extract dilution analysis (AEDA) (*12*) are focused on odorants that contribute to the flavor of Houjicha.

The Maillard reaction is the key pathway for the generation of both pyrazines and acrylamide. A theoretical investigation of a method to systematize complicated phenomena such as the Maillard reaction is important. By using a model system (asparagine and glucose), Ehling et al. (*13*) found that a linear relationship exists between the formation of acrylamide and alkylpyrazines during the initial stage of the Maillard reaction. Moreover, in a potato model, alkylpyrazines were formed at a rate similar to that of acrylamide at a heating temperature of 180 °C (*14*). Wedzicha et al. (*15*) proposed that there is a competition between the formation of alkylpyrazines and acrylamide. In one study, the addition of glycine successfully promoted the formation of several alkylpyrazines and limited acrylamide formation (*16*). Additionally, mechanisms of alkylpyrazine formation that involve added glycine have been reported (*17*). The Maillard reaction is complex. Thus, it would be interesting to know the relationship between the formation of acrylamide and alkylpyrazines in roasted tea products.

The high demand for tea is not only due to its desirable flavor but also because it contains many bioactive substances, mainly

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Table 1. Key Odorants (FD Factor ≥ 16) in Commercial Roasted Green Tea (Houjicha) and Roasted Product Processed at 160 °C for 30 min and at 180 °C for 15 min, Which Resulted in the Production of Desirable Houjicha Flavor

		LRI			FD factor[c]				
								Houjicha flavor was produced at	
peak	odorant[a]	GC-MS	GC-O	odor quality[b]	high[d]	medium[e]	low[f]	160 °C for 3 min[g]	180 °C for 15 min[g]
1	trimethyloxazole	1203	1205	caramel-like	16	<16	<16	<16	<16
2	ethylpyrazine	1345	1346	roasty, sweet	64	<16	<16	<16	<16
3	2-ethyl-6-methylpyrazine	1397	1397	roasty, sweet	16	32	16	32	32
4	2-ethyl-5-methylpyrazine	1404	1402	roasty, sweet	16	16	16	64	64
5	2,3,5-trimethylpyrazine	1418	1419	nutty	32	64	32	64	64
6	(*E*)-linalool oxide (furanoid)	1453	1456	floral	32	16	<16	<16	<16
7	2-ethyl-3,6-dimethylpyrazine	1458	1459	roasty, sweet	1024	512	32	512	512
8	2-ethyl-3,5-dimethylpyrazine	1474	1478	roasty, sweet	4096	2048	1024	4096	4096
9	(*Z*)-linalool oxide (furanoid)	1482	1482	floral	16	16	<16	<16	<16
10	2,3-diethyl-5-methylpyrazine	1505	1504	roasty, earthy	512	256	<16	256	512
11	linalool	1547	1547	floral, green	16	32	16	<16	<16
12	5-methylfurfural	1584	1583	burnt	<16	<16	16	<16	<16
13	unknown	1617	1620	cucumber-like	16	<16	<16	<16	<16
14	2-acetyl-1-ethylpyrrole	1657	1658	coffee liquor-like	128	64	128	128	128
15	furfuryl alcohol	1663	1664	earthy	16	32	<16	<16	<16
16	acetophenone	1670	1670	musty, pungent	<16	16	32	<16	<16
17	2-acetyl-3-methylpyrazine	1715	1716	nutty	128	128	128	64	64
18	unknown	1786	1788	sweet	16	32	64	64	64
19	methyl salicylate	1802	1802	antiseptic, pungent	64	<16	<16	128	256
20	unknown	1841	1845	sweet	16	16	<16	<16	<16
21	hexanoic acid	1850	1850	sweet, pungent	64	32	512	<16	128
22	guaiacol	1875	1875	burnt	128	64	512	<16	32
23	2-acetylpyrrole	1993	1994	caramel-like	<16	<16	16	<16	16
24	pyrrole-2-carboxaldehyde	2051	2052	burnt	64	256	2048	128	256
25	unknown	2076	2079	burnt	32	128	256	64	64
26	2-methoxy-4-vinylphenol	2219	2220	burnt, smoky	<16	<16	16	<16	<16
27	indole	2476	2476	animal-like	16	<16	<16	<16	<16

[a] The compound was identified by comparing it with the reference standard on the basis of the following criteria: linear retention indices (LRI) obtained by two methods, namely, GC-MS and GC-O; mass spectra obtained by MS; and odor quality as well as odor intensity perceived at the sniffing port. [b] Odor quality perceived at the sniffing port. [c] FD, flavor dilution factor. [d] High-priced Houjicha (2200 yen/100 g). [e] Medium-priced Houjicha (1000 yen/100 g). [f] Low-priced Houjicha (200 yen/100 g). [g] The desirable Houjicha flavor produced in the roasted product was evaluated by tea experts.

tea catechins. Thus, tea catechins are a very important factor for quality control in the tea industry (*18*). This research aims to optimize Houjicha processing by using roasting treatments to achieve low acrylamide content without compromising the quality. In the present paper, we determined the key odorants in Houjicha by using AEDA. Next, we investigated the effect of roasting treatments on the acrylamide, potent odorant, and tea catechin contents. It is expected that our results would greatly improve the industrial processing of Houjicha with regard to the mitigation of acrylamide formation without compromising the quality.

MATERIALS AND METHODS

Materials. To determine the key odorants, three commercial products, namely, high- (2200 yen/100 g), medium- (1000 yen/100 g), and low-priced (200 yen/100 g) Houjicha, were purchased from tea stores in Shizuoka Prefecture, Japan, in August 2007. For assessing the effect of the roasting condition on the acrylamide, potent odorant, and tea catechin contents, tea leaves of the cultivar 'Yabukita' were plucked on May 4, 2006, in Shizuoka Prefecture, Japan. The tea leaves were processed by drying at temperatures below 80 °C, and the weight of the product obtained was approximately 21 kg. The dry matter (DM) content of the tea material was 93.75%.

Chemicals. For determining the linear retention indices (LRI) of the odorants, *n*-alkanes (n = 6–26) were purchased from Tokyo Chemical Industry Co. Ltd., Tokyo, Japan. The odorants listed in **Table 1** were used to prepare calibration standards for quantification and identification and yielded peaks 1, 2, 5, 10–12, 14–17, 19, 21–24, 26, and 27 (Tokyo Chemical Industry). Furthermore, linalool oxide that yielded peaks 6 and 9 was also purchased from Tokyo Chemical Industry. 2-Ethyl-5(6)-methylpyrazine (peaks 3 and 4) and 2-ethyl-3,5(6)-dimethylpyrazine (peaks 7 and 8) were obtained from Sigma-Aldrich Co. Ltd. (Tokyo, Japan). Analytical grade diethyl ether purchased from Tokyo Chemical Industry was used for the extraction of odorants. Diethyl ether was refined by the liquid–liquid extraction method using a 10% w/v sodium hydroxide solution and distillation at 40 °C under atmospheric pressure before use. The chemicals used for acrylamide (*10*) and tea catechin analysis (*18*) in our laboratory have been described previously.

Roasting Condition Test of the Tea Material. The tea material weighing 100 g was placed on an aluminum plate (310 mm × 180 mm) and roasted at 160 and 180 °C. At each temperature, roasting was carried out for 5–40 min at intervals of 5 min in a laboratory oven (DK-660; Yamato Scientific Co. Ltd., Tokyo, Japan). The roasted product was then placed in a desiccator with 300 mL of silica gel to cool the sample to room temperature (20–25 °C) and then stored in a refrigerator at −30 °C until use. The roasting treatment was repeated three times. Five tea experts trained in sensory evaluation determined the roasting condition that produced the desirable Houjicha flavor.

Tea Infusion. Tea infusion was prepared as follows, according to standardized methods (*19*) established by the Japan Science and Technology Agency. The roasted tea leaves (10 g) were weighed in a beaker (1 L), and 650 mL of hot water (90 °C) was added. The mixture was left to stand for 1 min in a hot water bath (KZ-1000T; National Co. Ltd., Tokyo, Japan) at 90 °C. The mixture was filtered through a tea strainer (mesh size < 1 mm^2), and the filtrate was cooled to room temperature in an ice bath. The filtrate was centrifuged at 20000 rpm for 10 min at 20 °C, and the supernatant obtained was used as the analyte. For quantification, this treatment was repeated three times.

Analysis of Odorants. Tea infusion (0.2 L) was distilled under reduced pressure (40 °C, 2.0 kPa). The steam distillate was spiked with ethyl decanoate, which was used as the internal standard. The distillate was saturated with sodium chloride and then extracted in triplicate with diethyl ether (100 mL each time). The extract was dried over anhydrous sodium sulfate, and the solvent was removed by a rotary evaporator to obtain approximately 1 mL of concentrate at 40 °C under atmospheric pressure. Further concentration to 50 μL was conducted with a nitrogen stream. The concentrate thus obtained was used for quantitation.

The gas chromatography (GC) system (Hewlett-Packard 5890 series II unit; Hewlett-Packard, Palo Alto, CA) equipped with a DB-Wax capillary column (60 m × 0.25 mm i.d.; film thickness, 0.25 μm; J&W Scientific, Folsom, CA) was operated in the splitless injection mode. The concentrate (2 μL) was injected into the column. The injection temperature was set at 230 °C. The oven temperature was maintained at 40 °C for 1 min, increased by 4 °C/min to 220 °C, and maintained at this temperature for 45 min. Helium was used as the carrier gas under a column head pressure of 100 kPa and a flow rate of 1 mL/min. The compounds were quantified on the basis of total ion chromatograms (TICs) obtained using a mass spectrometer (SX102H; JEOL, Tokyo, Japan) in the electron impact ionization mode at 70 eV. The source temperature ranged between 170 and 200 °C. A scan range of m/z 33–400 at 2.05 scans s^{-1} was employed. The n-alkanes (C_6–C_{26}) were also analyzed under the same conditions to obtain the LRI value of the components by gas chromatography–mass spectrometry (GC-MS) analysis.

Quantification of Odorants. The quantities of the components were estimated by comparing their peak areas on the TICs with that of ethyl decanoate. The internal standard method was employed for nine calibration solutions (200 mL each) of each compound that contained between 0.15 and 250 μg of the compound and 200 μg of the internal standard ethyl decanoate. The standard solution was saturated with sodium chloride and then extracted in triplicate with diethyl ether (100 mL for each extraction). The extract was dried over anhydrous sodium sulfate, and the solvent was then concentrated to approximately 50 μL. Analytical recovery was established by spiking a distillate of tea infusion with a known amount of pure standard and comparing this to the amount recovered after extraction. Alkylpyrazines with the same molecular mass were assumed to have identical analytical recovery, and the calculated quantities were adjusted to accommodate the observed recovery.

Gas Chromatography−Olfactometry (GC-O). A Hewlett-Packard 5890 gas chromatograph system equipped with a flame ion detector (FID) and a DB-Wax capillary column (60 m × 0.25 mm i.d.; film thickness = 0.25 μm; J&W Scientific) was operated in the splitless injection mode. Helium was used as the carrier gas under a column head pressure of 100 kPa and flow rate of 1 mL/min. The oven temperature was maintained at 40 °C for 1 min, increased by 3 °C/min to 220 °C, and maintained at this temperature for 1 h. The injection and detection port temperatures were both set at 230 °C. The GC eluate of the odor concentrate was separated to the FID and sniffing port immediately in front of the detector port (split ratio = 1:1). Moist air was pumped into the sniffing port at 100 mL/min. The n-alkanes (C_6–C_{26}) were also analyzed under the same conditions to obtain the LRI values by the GC-O analysis.

AEDA. Tea infusion (3.0 L) was distilled under reduced pressure (40 °C, 2.0 kPa). Steam distillate was obtained and then extracted with diethyl ether. The extract was concentrated to 50 μL. The dilution (2 μL) was injected into the GC-O system. The concentrate was diluted stepwise with diethyl ether to 2^n. The odorants were then detected by GC-O and FID. The flavor dilution (FD) factors of the odorants were determined by AEDA. The FD factor and odor quality were confirmed by two trained panelists.

Identification of Odorants. The components were identified by comparing their LRI, mass spectra, and odor quality to those of pure compounds. The LRI of the odorants was calculated from the retention time of the n-alkanes by using GC-MS and GC-O.

Analysis of Acrylamide. Acrylamide was analyzed according to our previously described method (*10*) in which the analyte was detected as a dibromo derivative (2,3-dibromopropionamide) by GC-MS with the following modifications. The internal standard ([1,2,3-$^{13}C_3$]acrylamide) was added to 25 mL of the tea infusion in a volumetric flask. The mixture (3 mL) was passed through 3 mL of IR-120B ion-exchange resin (Rohm and Haas, Philadelphia, PA) to remove mainly tea catechins from the mixture. The resin was pre-equilibrated with water for >24 h prior to use. Next, the eluate (2 mL) was applied to a solid phase extraction (SPE) cartridge (Isolute Multimode, 500 mg; International Sorbent Technology, Hengoed, Mid Glamorgan, U.K.) that was preconditioned with methanol (1 mL) and water (2 mL). The first SPE eluate (0.5 mL) was discarded, and the next eluate (2 mL) was used for bromination. We confirmed that the SPE eluate used for bromination contained no tea catechins that inhibited acrylamide bromination. The latter procedures and GC-MS conditions were followed as described in our previous study (*10*).

Analysis of Tea Catechins. The internal standard catechol was weighed (69.4 mg), transferred into a 100 mL volumetric flask containing ascorbic acid (176 mg), and dissolved in 90 mL of water by sonication for 1 h at 20–25 °C; the volume was made up to 100% with water. The internal standard solution (1 mL) was added to tea infusion (2 mL) and mixed by shaking for 1 min. The mixture was diluted with water to achieve a volume of 10 mL and passed through a 0.45 μm filter disk (Advantec, Tokyo, Japan) prior to injection. The high-performance liquid chromatography−UV detector conditions were followed as described in our previous study (*18*).

RESULTS AND DISCUSSION

Potent Odorants and Their Formation in Houjicha. The desirable flavor for Houjicha is mainly roasty. The components that contribute to this characteristic odor were separated by steam distillations performed under reduced pressure, and the steam distillates were extracted using diethyl ether. AEDA was used for the objective determination of the components that contribute to the characteristic odor of Houjicha. We used three types of Houjicha (high-, medium-, and low-priced) for AEDA. In addition, the desirable Houjicha flavor was obtained in the preliminary sensory experiments by roasting at 160 °C for 30 min and at 180 °C for 15 min (as evaluated by a panel of five tea experts). Therefore, these two roasted products were also used for AEDA. AEDA revealed 27 odor-active peaks with FD factors of ≥16, as shown in **Table 1**. Stepwise dilution of the odor concentrate resulted in three peaks with high odor potencies (FD factors in the range of 1024–4096). The potent odorants indicated by these peaks showed roasty (peak 7), sweet (peak 8), and burnt (peak 24) odor qualities. The three odorants indicated by these peaks had high odor potencies and were identified as 2-ethyl-3,6-dimethylpyrazine (peak 7), 2-ethyl-3,5-dimethylpyrazine (peak 8), and pyrrole-2-carboxaldehyde (peak 24). When high-priced Houjicha was subjected to AEDA, the FD factors of peaks 7, 8, and 24 were 1024, 4096, and 64, respectively. In contrast, when low-priced Houjicha was analyzed, the FD factors of peaks 7, 8, and 24 were 32, 1024, and 2048, respectively. Thus, the high FD factors of 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine (roasty and sweet odors, respectively) were detected along with the low FD factor of pyrrole-2-carboxaldehyde (burnt odor) in high-priced Houjicha. Moreover, in the roasted test products, 2-ethyl-3,5-dimethylpyrazine had an extremely high FD factor of 4096; these products had desirable flavors as evaluated by the tea experts (**Table 1**). Hence, on the basis of these results, we identified 2-ethyl-3,5-dimethylpyrazine as the most important odorant in Houjicha.

Roasting treatments at 160 °C for 30 min and 180 °C for 15 min yielded the desirable Houjicha flavor. The level of potent odorants 2-ethyl-3,6-dimethylpyrazine (peak 7) and 2-ethyl-3,5-dimethylpyrazine (peak 8) sharply increased until 10 min of roasting at 180 °C and then remained stable (**Figure 1**). Alkylpyrazines are stable products of the Maillard reaction (*14*). Therefore, their levels remained stable with continued heating. The levels of 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine also increased slowly at a roasting temperature of 160 °C. After 30 min of roasting at 160 °C, the most important odorant, 2-ethyl-3,5-dimethylpyrazine, was formed;

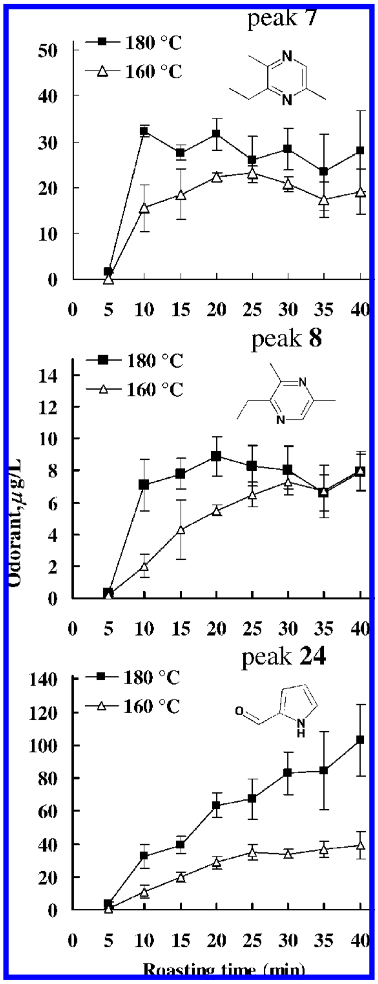

Figure 1. Effects of roasting temperature and time on the contents of the potent odorants 2-ethyl-3,6-dimethylpyrazine (peak 7), 2-ethyl-3,5-dimethylpyrazine (peak 8), and pyrrole-2-carboxaldehyde (peak 24) in tea infusion. Tea leaves weighing 100 g were placed on an aluminum plate and roasted in a laboratory oven. The roasting treatment and preparation for tea infusion were repeated three times. Each data value is expressed as mean ± standard deviation (SD) ($n = 9$).

its level was 7.3 μg/L, which was approximately the same as the amount formed by roasting for 15 min at 180 °C (**Figure 1**). The amount of 2-ethyl-3,6-dimethylpyrazine formed by roasting at 180 °C for 15 min was slightly higher than that formed by roasting at 160 °C for 30 min (**Figure 1**). However, this small difference between the levels of 2-ethyl-3,6-dimethylpyrazine in the two treatments did not appear to greatly influence the Houjicha flavor because the same FD factor was detected by both treatments, as shown in **Table 1**. Therefore, the potent odorants in Houjicha were formed at levels adequate to influence the Houjicha flavor, even at a comparatively low temperature of 160 °C. AEDA revealed that pyrrole-2-carboxaldehyde (peak 24), which has a burnt odor, in low-priced Houjicha has a high FD factor. This odorant increased with continued roasting at both 180 and 160 °C (**Figure 1**). The maximum amount of this odorant was formed by roasting at 180 °C and was approximately 2.7 times that formed by roasting at 160 °C. As mentioned above, the potent odorants 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine were formed at levels adequate to influence the Houjicha flavor, even at a low roasting temperature of 160 °C, whereas pyrrole-2-carboxaldehyde with a burnt odor did not contribute greatly to the flavor.

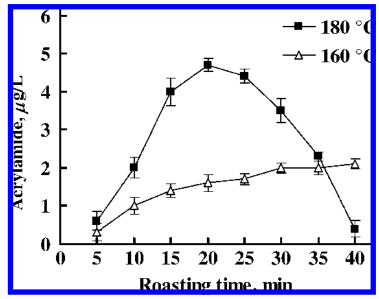

Figure 2. Effects of roasting temperature and time on the acrylamide content in tea infusion. Tea leaves weighing 100 g were placed on an aluminum plate and roasted in a laboratory oven. The roasting treatment and preparation for tea infusion were repeated three times. Each data value is expressed as mean ± SD ($n = 9$).

On the basis of the AEDA results, 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine have been characterized as important flavor contributors to heated foodstuffs (*20–26*). These pyrazines were detected among the potent contributors due to their extremely low odor threshold as compared to other pyrazines (*26*). A pyrazine with a wide variety of alkyl substituents would have many potential formation pathways (*17, 27, 28*). The possible formation pathway of 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine involving alanine has been reported previously (*17, 27, 28*). Briefly, 2,6-dimethyl- and 2,5-dimethyldihydropyrazine are formed from aminoacetaldehyde, which is derived from the Strecker reaction of methylglyoxal. Acetaldehyde, which is derived from the Strecker reaction of alanine, combines with dimethyldihydropyrazine, finally resulting in 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine. The amino acid glycine has been shown to promote the formation of 2-ethyl-3,5-dimethylpyrazine (*17*). It was observed that 2-ethyl-3,6-dimethylpyrazine was formed approximately 4 times more than 2-ethyl-3,5-dimethylpyrazine (**Figure 1**). In addition, the tea material contained more alanine than glycine (below the detection limit of 3 μg/g) (*10*). Therefore, the potent odorants 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine in Houjicha are mainly formed from alanine without the glycine promotion of their formation in the tea material.

Acrylamide Content. We have previously reported that acrylamide is formed during roasting (*10*). Here, its formation by roasting at 160 and 180 °C is reported in detail. The process of acrylamide formation was similar to that reported in our previous study. At the roasting temperature of 160 °C, the acrylamide content in tea infusion slowly increased to 2.1 μg/L (**Figure 2**). The maximum amount of acrylamide in tea infusion was 4.7 μg/L; this level was attained when the tea material was roasted at 180 °C for 20 min. The condition for maximum acrylamide formation was different from that reported in our previous study (180 °C for 10 min) (*10*). This may be because the initial DM level of the tea material used in this study (93.75% DM) was lower than that of the tea material used in the previous study (94.95% DM). The desirable Houjicha flavor, which was evaluated by tea experts, was obtained by the roasting treatments of 160 °C for 30 min and 180 °C for 15 min, during which 2.0 and 4.0 μg/L of acrylamide, respectively, were formed. Thus, the roasting temperature of 160 °C is strongly recommended for mitigation of acrylamide without compromising the sensory quality.

Tea Catechin Contents. The demand for tea has increased due to human preferences and health concerns. Tea contains many bioactive substances and is particularly rich in polyphenol

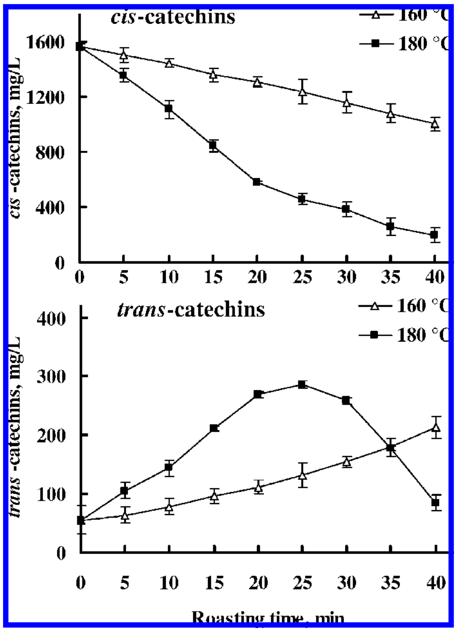

Figure 3. Effects of roasting temperature and time on the contents of *cis*-catechins (EGCg, ECg, EGC, and EC) and *trans*-catechins (GCg, Cg, Gc, and C) in tea infusion. Tea leaves weighing 100 g were placed on an aluminum plate and roasted in a laboratory oven. The roasting treatment and preparation for tea infusion were repeated three times. Each data value is expressed as mean ± SD ($n = 9$).

compounds. The predominant polyphenols in tea are catechins. We tested for the presence of tea catechins, including epigallocatechin gallate (EGCg), epicatechin gallate (ECg), epigallocatechin (EGC), epicatechin (EC), gallocatechin gallate (GCg), catechin gallate (Cg), gallocatechin (Gc), and catechin (C), in tea infusion.

Figure 3 shows the changes in *cis*-catechins (EGCg, ECg, EGC, and EC) and *trans*-catechins (GCg, Cg, Gc, and C). The main tea catechins in tea infusion are *cis*-catechins, and the levels of these catechins decreased linearly during roasting. The *trans*-catechins increased when roasting at 160 °C was continued for 40 min. Although the amount of *trans*-catechins also increased with roasting at 180 °C, the levels peaked after 25 min of roasting and then subsequently decreased. During the heat treatment, some catechins undergo isomerization at the C-2 position of flavan-3-ol (*29*). For example, EGCg, ECg, EGC, and EC are converted to GCg, Cg, Gc, and C, respectively. Hence, epimerization also occurs during the roasting treatment. The deep roasting treatment induced a decrease in the levels of tea catechins; these levels were easily calculated from the data shown in **Figure 3**. This decrease may be due to oxidation, decomposition, and polymerization (*30*). The desirable flavor of Houjicha is obtained by roasting treatments of 160 °C for 30 min and 180 °C for 15 min; these treatments yielded 1316 and 1057 mg/L of tea catechins, respectively.

In conclusion, the roasting treatment of 160 °C for 30 min is strongly recommended for Houjicha processing for acrylamide mitigation, formation of potent odorants, and inhibition of degradation of tea catechins without compromising the sensory quality.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; DM, dry matter; LRI, linear retention indices; GC, gas chromatography; TIC, total ion chromatogram; GC-MS, gas chromatography–mass spectrometry; GC-O, gas chromatography−olfactometry; FID, flame ion detector; FD factor, flavor dilution factor; SPE, solid phase extraction; EGCg, epigallocatechin gallate; ECg, epicatechin gallate; EGC, epigallocatechin; EC, epicatechin; GCg, gallocatechin gallate; Cg, catechin gallate; Gc, gallocatechin; C, catechin; SD, standard deviation.

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