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Potent Inhibitory Effects of Black Tea Theaflavins on Off-Odor Formation from Citral

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The present study was conducted to investigate the ability of black tea theaflavins to inhibit the off-odor formation from citral under acidic aqueous conditions. Acidic buffer solutions (pH 3.0) containing citral (10 mg/L) and an inhibitor (0−5 mg/L) were stored at 40 °C for 2 weeks. The formation of possible off-odorants *p*-cresol and *p*-methylacetophenone in the citral solutions was monitored by high-performance liquid chromatography. A mixture of the theaflavins showed inhibitory effects on the formation of both *p*-cresol and *p*-methylacetophenone with 50% inhibitory concentrations (IC_{50}) of 0.18 and 0.10 mg/L, respectively. Individual theaflavins and a structurally related compound, purpurogallin, also inhibited the formation of both off-odorants, with the lowest IC_{50} values for theaflavin 3,3′-digallate (0.17 and 0.06 mg/L for *p*-cresol and *p*-methylacetophenone, respectively). On the other hand, a mixture of green tea catechins and its major constituent, (−)-epigallocatechin gallate, showed relatively high IC_{50} values for the formation of *p*-methylacetophenone (1.29 and 1.28 mg/L, respectively) and showed no inhibitory effect on the formation of *p*-cresol. The results of the sensory evaluation showed that the off-odor intensity of the stored citral solution was significantly decreased by the addition of the theaflavin mixture at concentrations of 0.5 mg/L and above. In addition, the calculation of the odor activity values (OAVs) for the volatile compounds detected by a gas chromatographic analysis indicated that the total OAVs of the major volatile compounds in the citral solution were significantly decreased by the addition of the theaflavins.

KEYWORDS: Citral; off-odors; inhibition; black tea; theaflavins; *p*-cresol; *p*-methylacetophenone; odor activity values

INTRODUCTION

The stability of citral (a mixture of geranial and neral) under acidic aqueous conditions has received much attention in the field of flavor chemistry (*1*−*7*). Under such conditions, citral is easily degraded by a series of cyclization and oxidation reactions to form a variety of degradation products (*1*). Consequently, not only is the fresh lemon-like odor of citral lost, but undesirable off-odors develop. Among the degradation products from citral, *p*-cymene, α,*p*-dimethylstyrene, and *p*-cymen-8-ol were postulated to be responsible for the undesirable off-odors (*2*, *3*). However, in later studies using aroma extract dilution analyses, *p*-cresol and *p*-methylacetophenone were suggested to be more responsible for the off-odors from citral than the formerly proposed compounds (*4*, *5*). Both *p*-cresol and *p*-methylacetophenone showed low odor thresholds (0.3−1.0 and 2.7−10.8 ng/L in air, respectively), and their odor characters were described as "phenolic" and "bitter-almond-like," respectively (*4*, *5*).

Antioxidants have been used to inhibit the off-odor formation from citral under acidic aqueous conditions (*2*, *3*, *6*, *7*). In earlier studies in which *p*-cymene, α,*p*-dimethylstyrene, and *p*-cymen-8-ol were postulated to be the potent off-odorants (*2*, *3*), only isoascorbic acid was reported to inhibit the formation of α,*p*-dimethylstyrene and *p*-cymen-8-ol (*3*). Recently, Liang et al. (*6*) reported that plant extracts such as green tea and black tea extracts inhibited the formation of the other possible off-odorants, that is, *p*-cresol and *p*-methylacetophenone. However, in our previous study (*7*), individual green tea catechins such as (−)-epigallocatechin gallate showed strong inhibitory effects on the formation of *p*-methylacetophenone, but these catechins did not inhibit the formation of *p*-cresol and, in some instances, promoted it. Therefore, the reason green tea extracts can inhibit the formation of *p*-cresol as shown by Liang et al. (*6*) might be due to constituents other than the catechins. On the other hand, theaflavins, major phenolic constituents of black tea, have not been fully evaluated for their ability to inhibit the off-odor formation from citral. Although a black tea extract containing theaflavins was reported to inhibit the formation of *p*-cresol and *p*-methylacetophenone (*6*), the effects of pure theaflavins on the formation of these off-odorants have not been reported.

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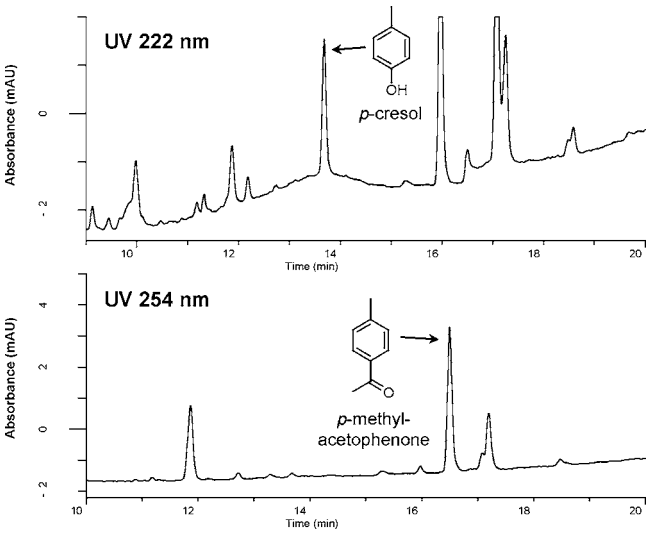


Figure 1. HPLC analysis of *p*-cresol and *p*-methylacetophenone formed from citral (10 mg/L) stored under acidic aqueous conditions (pH 3) at 40 °C for 2 weeks.

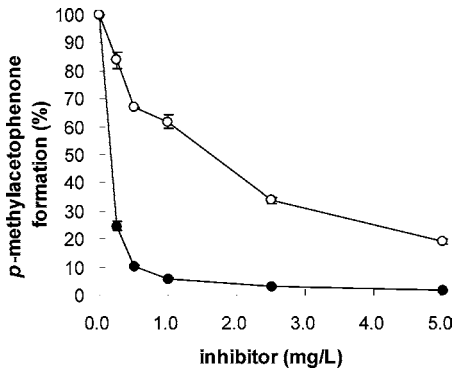


Figure 2. Dose-dependent effects of mixtures of tea polyphenols on the formation of *p*-methylacetophenone from citral (10 mg/L) stored under acidic aqueous conditions (pH 3) at 40 °C for 2 weeks: theaflavin mixture (●); catechin mixture (○). Each value is expressed as the mean ± standard deviation of triplicate samples.

The objectives of the present study were to investigate the effects of black tea theaflavins on the formation of the possible off-odorants *p*-cresol and *p*-methylacetophenone from citral under acidic aqueous conditions and to verify the off-odor-reducing ability of the theaflavins by a sensory evaluation and by using the concept of odor activity values (OAVs) (*8*).

MATERIALS AND METHODS

Materials. Citral was purchased from Polarome International (Jersey City, NJ). A crude mixture of theaflavins [extracted from black tea; total theaflavins, 92.2 area % (measured by HPLC-UV analysis)], a crude mixture of catechins (extracted from green tea; total catechins, 88.2 wt %), and (−)-epigallocatechin gallate were purchased from Funakoshi (Tokyo, Japan). Theaflavin, theaflavin 3-gallate, theaflavin 3′-gallate, and theaflavin 3,3′-digallate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Purpurogallin was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The analytical standards for *p*-cresol, *p*-methylacetophenone, *p*-cymene, α,*p*-dimethylstyrene, *p*-cymen-8-ol, and 8-hydroperoxy-*p*-cymene were obtained as previously described (*7*). All other chemicals used in this study were of the highest grade commercially available.

Sample Preparation. A citral solution was prepared by dissolving 10 mg/L of citral in a pH 3 buffer (0.1 M citric acid−0.2 M sodium hydrogen phosphate), and an inhibitor (one of the mixtures of tea polyphenols or one of the individual phenolic compounds) was added to the citral solution at various concentrations (0, 0.25, 0.5, 1.0, 2.5, and 5.0 mg/L). Aliquots (3 × 18 mL) of the stock solutions were transferred into 20-mL crimp-top glass vials with PTFE/silicone septa. Other aliquots (3 × 100 mL) of the same solutions were transferred into 100-mL screw-top glass vials with Teflon-faced rubber liners. The headspace volume in the sample vials, which can be measured by filling the vials with the sample solution without a headspace, was 3 mL for the 20-mL vials and 17 mL for the 100-mL vials (i.e., ∼17% of the sample volume for both types of vials). All samples were stored in a dark incubator at 40 °C for 2 weeks. After storage, the samples in the 20-mL vials were subjected to high-performance liquid chromatography (HPLC) and to the dissolved oxygen measurement. The samples in the 100-mL vials were extracted with dichloromethane as previously described (*7*) and then subjected to a gas chromatographic (GC) analysis. For the sensory evaluation, the samples containing the theaflavin mixture at different concentrations (0, 0.1, 0.5, and 2.5 mg/L) were prepared and stored in the 100-mL vials as already described.

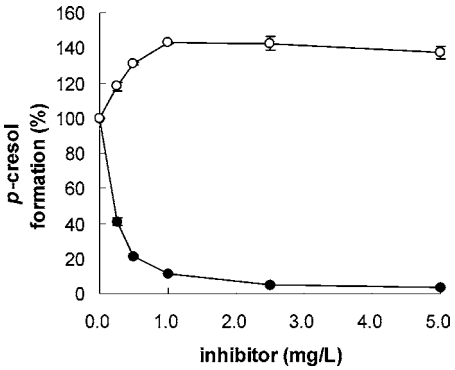


Figure 3. Dose-dependent effects of mixtures of tea polyphenols on the formation of *p*-cresol from citral (10 mg/L) stored under acidic aqueous conditions (pH 3) at 40 °C for 2 weeks: theaflavin mixture (●); catechin mixture (○). Each value is expressed as the mean ± standard deviation of triplicate samples.

Table 1. Estimated IC_{50} Values of Mixtures of Tea Polyphenols

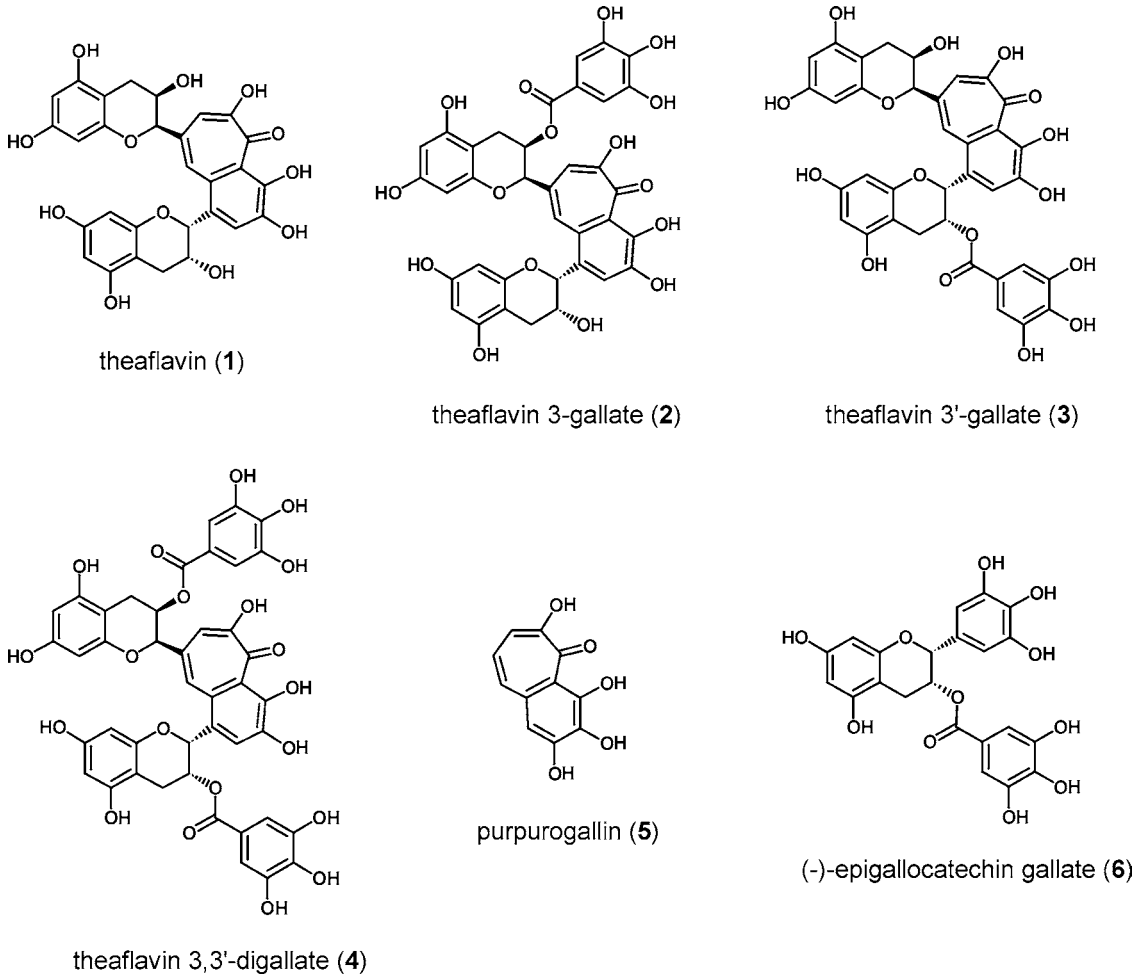
	IC_{50}[a] (mg/L)	
off-odorant	theaflavin mixture	catechin mixture
p-methylacetophenone	0.10 (0.01)	1.29 (0.11)
p-cresol	0.18 (0.01)	nd[b]

[a] IC_{50} values were calculated by nonlinear regression of the dose-dependent inhibition data. Asymptotic standard errors are shown in parentheses. [b] Not determined.

Measurement of Dissolved Oxygen (DO). The DO was measured for the samples stored in the 20-mL vials using a CHEmets DO test kit K-7512 (CHEMetrics, Inc., Claverton, VA). For the blank measurements, nitrogen was bubbled through the solution for 5 min via a needle inserted into the sealed vials also equipped with another needle as an outlet. All measurements were conducted right after the vials had been opened. The stored citral solutions constantly showed a DO concentration of 8 mg/L regardless of the addition of the theaflavin mixture (0−5 mg/L), whereas the blank measurements showed a DO concentration of <1 mg/L, suggesting that the DO concentrations were in equilibrium with the headspace in the vials and that the addition of the theaflavins at these doses did not influence the DO concentration at a measurable level.

HPLC. An Agilent 1100 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA) and a Capcell Pak C_{18} MG column (250 mm × 4.6 mm i.d.; particle size, 5 μm; Shiseido, Tokyo, Japan) were used. The operating conditions were as follows: column oven temperature, 40 °C; mobile phase, a linear gradient from 100% solvent A (10% acetonitrile, pH 2.5 adjusted with phosphoric acid) to 100% solvent B (acetonitrile) in 25 min, held at 100% B for 2 min; flow rate, 1 mL/min; and injection volume, 20 μL. All samples were filtered though a 0.45-μm PTFE membrane filter

Chart 1. Chemical Structures of Individual Phenolic Compounds



(DISMIC-13HP, Toyo Roshi, Tokyo, Japan) prior to injection. Wavelengths of 222 and 254 nm were used for the quantification of *p*-cresol and *p*-methylacetophenone, respectively, and the UV spectra (wavelengths from 200 to 400 nm) plus retention time were used for their identification.

GC and Gas Chromatography−Mass Spectrometry (GC-MS). The major volatile compounds extracted from the stored citral solutions were analyzed by GC-MS, and their quantities were determined by GC, as previously described (*7*). The response factors for all compounds to the flame ionization detector (FID) were assumed to be the same as that of the internal standard (*n*-pentadecane), and for that reason, the quantities determined by the GC measurements are tentative values.

Sensory Evaluation. The stored citral solutions containing the theaflavin mixture at different concentrations were evaluated for their off-odor intensity. A standard procedure for test design and sample presentation was used (*9*). The samples (30 mL of each in a 100-mL plastic cup) were randomized and simultaneously presented to each panelist. The panelists ($n = 17$) were asked to sniff the headspace of the samples and rate the intensity of the overall off-odor using a seven-point scale ranging from 1 (= absent) to 7 (= very strong). The citral solution without the theaflavins was presented as a reference of the off-odor intensity (intensity 6) and also used as a blind control in a series of test samples. The data were analyzed by a one-way analysis of variance using SPSS 13.0 (SPSS Inc., Chicago, IL). Significant differences between pairs of samples were determined using the Bonferroni test. Differences of $p < 0.05$ were considered to be significant.

Calculation of IC_{50} Values. The dose-dependent inhibition curves obtained for each inhibitor, such as shown in **Figures 2** and **3**, were fitted to a four-parameter logistic equation: $Y = (A - B)/[1 + (X/C)^D] + B$, where X is the concentration of inhibitor (mg/L), Y is the relative amount (%) of *p*-cresol or *p*-methylacetophenone, A is the maximum value of Y (= 100%), B is the minimum value of Y (= 0%), C is the concentration of inhibitor giving 50% inhibition (IC_{50}, mg/L), and D is the curve slope at the inflection point. The parameters C (IC_{50}) and D were determined by a least mean square fit using the nonlinear regression procedure of SPSS 13.0 (SPSS Inc.).

Determination of Odor Threshold of *p*-Cymen-8-ol. *p*-Cymen-8-ol (Bedoukian Research, Danbury, CT), which contained a small amount of *p*-methylacetophenone as an impurity, was purified by silica gel column chromatography using dichloromethane as the eluent. Purification was repeated to completely remove the impurity. The orthonasal detection threshold in water was determined according to ASTM procedure E679-04, an ascending forced-choice method of limits (*10*). Samples containing *p*-cymen-8-ol were prepared by spiking solutions of *p*-cymen-8-ol (in 50 wt % aqueous ethanol) into water at six final concentrations (1.1, 3.3, 10, 30, 90, and 270 mg/L). The controls were prepared by spiking the corresponding amounts of 50 wt % aqueous ethanol into water. Three samples (two controls and one containing *p*-cymen-8-ol, 30 mL of each in a 100-mL plastic cup) were presented at ascending concentration levels of *p*-cymen-8-ol. Panelists ($n = 21$) were asked to sniff the headspace of the samples and choose the odd sample from the three. The best-estimate threshold for each panelist was the geometric mean of the highest concentration missed and the next higher concentration tested. The group threshold was calculated as the geometric mean of the individual best-estimate thresholds.

RESULTS AND DISCUSSION

Inhibitory Activity of Mixtures of Tea Polyphenols on the Formation of *p*-Cresol and *p*-Methylacetophenone from Citral. We first compared a mixture of theaflavins and that of catechins (extracted from black tea and green tea, respectively) for their ability to inhibit the formation of *p*-cresol and

Table 2. Estimated IC_{50} Values of Individual Phenolic Compounds **1**–**6**[a]

	IC_{50}[b] (mg/L)					
off-odorant	**1**	**2**	**3**	**4**	**5**	**6**
p-methylacetophenone	0.20 (0.01)	0.15 (0.02)	0.23 (0.01)	0.06 (0.01)	0.17 (0.01)	1.28 (0.12)
p-cresol	0.33 (0.02)	0.20 (0.00)	0.42 (0.01)	0.17 (0.02)	0.51 (0.03)	nd[c]

[a] Numbers represent the following compounds: **1**, theaflavin; **2**, theaflavin 3-gallate; **3**, theaflavin 3′-gallate; **4**, theaflavin 3,3′-digallate; **5**, purpurogallin; **6**, (–)-epigallocatechin gallate. [b] IC_{50} values were calculated by nonlinear regression of the dose-dependent inhibition data. Asymptotic standard errors are shown in parentheses. [c] Not determined.

p-methylacetophenone from citral under acidic aqueous conditions. The relative amounts of each off-odorant in the stored citral solutions were determined by HPLC analysis (**Figure 1**), and the results were plotted against the concentration of the tea polyphenols (**Figures 2** and **3**). Both the theaflavin mixture and the catechin mixture inhibited the formation of *p*-methylacetophenone in a dose-dependent manner, but the theaflavin mixture showed much higher activity than the catechin mixture. In addition, the theaflavin mixture showed a strong inhibitory effect on the formation of *p*-cresol, whereas the catechin mixture promoted it at all concentrations tested. In our previous study under similar experimental conditions (*7*), some of the individual catechins found in green tea, such as (−)-epigallocatechin gallate, also showed promoting effects on the formation of *p*-cresol. From the dose-dependent inhibition curves obtained for each polyphenol mixture, the IC_{50} values were calculated by nonlinear regression using a logistic model (**Table 1**). The *p*-methylacetophenone IC_{50} of the theaflavin mixture and that of the catechin mixture were determined to be 0.10 and 1.29 mg/L, respectively. This indicates that the inhibitory activity of the theaflavin mixture on the formation of *p*-methylacetophenone is ∼10 times stronger than that of the catechin mixture. In addition, the *p*-cresol IC_{50} of the theaflavin mixture was determined to be 0.18 mg/L, whereas the catechin mixture showed no inhibitory effect on the formation of *p*-cresol.

Inhibitory Activity of Individual Phenolic Compounds on the Formation of *p*-Cresol and *p*-Methylacetophenone from Citral. To obtain further knowledge of the inhibitory activity of the theaflavins, individual phenolic compounds including the four major theaflavins found in black tea [theaflavin (**1**), theaflavin 3-gallate (**2**), theaflavin 3′-gallate (**3**), and theaflavin 3,3′-digallate (**4**)], purpurogallin (**5**), and (−)-epigallocatechin gallate (**6**) were used as inhibitors (**Chart 1**). Compound **5** is an aglycone of several glycosides found in the nutgalls of oak barks (*11*). This compound has a benzotropolone ring similar to the theaflavins. Compound **6** was used as a reference compound with a known inhibitory effect (*7*). On the basis of the same procedure as described for the mixtures of tea polyphenols, the IC_{50} values of these phenolic compounds were determined (**Table 2**). The individual theaflavins **1**−**4** and compound **5** inhibited the formation of *p*-methylacetophenone with IC_{50} values ranging from 0.06 to 0.23 mg/L. In addition, all of the compounds **1**−**5** inhibited the formation of *p*-cresol with IC_{50} values ranging from 0.17 to 0.51 mg/L. In contrast to compounds **1**−**5**, (−)-epigallocatechin gallate (**6**) showed a relatively high IC_{50} value for the formation of *p*-methylacetophenone (1.28 mg/L) and showed no inhibitory effect on the formation of *p*-cresol. These results suggest that the benzotropolone moiety common to compounds **1**−**5** might play a major role in their inhibitory activity. Among the individual theaflavins **1**−**4**, the IC_{50} values for both the *p*-methylacetophenone and *p*-cresol formation increased in the following order: theaflavin 3,3′-digallate (**4**) < theaflavin 3-gallate (**2**) < theaflavin (**1**) < theaflavin 3′-gallate (**3**). This suggests that the 3-galloyl moiety might play a more important role than the 3′-galloyl moiety in enhancing the inhibitory activity of theaflavins.

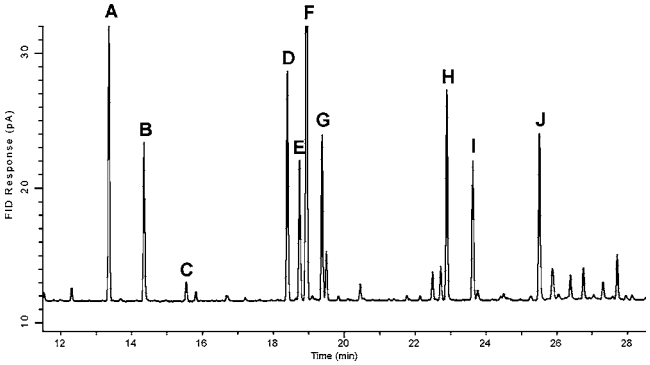


Figure 4. GC analysis of the volatile compounds extracted from the acidic aqueous solution (pH 3.0) of citral (10 mg/L) stored at 40 °C for 2 weeks. Peaks: A, *p*-cymene; B, *p*-cresol; C, α,*p*-dimethylstyrene; D, *p*-mentha-1,5-dien-8-ol; E, *p*-methylacetophenone; F, *p*-cymen-8-ol; G, *p*-mentha-1(7),2-dien-8-ol; H, *trans*-*p*-menth-2-ene-1,8-diol; I, *cis*-*p*-menth-2-ene-1,8-diol; J, 8-hydroperoxy-*p*-cymene.

Effects of Theaflavins on the Formation of Major Degradation Products from Citral. The results obtained indicate that theaflavins are potent inhibitors of *p*-cresol and *p*-methylacetophenone formed from citral under acidic aqueous conditions. To obtain information on other possible off-odorants from citral, the volatile compounds extracted from the stored citral solutions were analyzed by GC and GC-MS (**Figure 4**). The major products from citral detected by the GC analysis were divided into two groups. One was the acid-catalyzed reaction products, such as *p*-cymene (peak A), *p*-menthadien-8-ols (peaks D and G), and *p*-menth-2-ene-1,8-diols (peaks H and I). The formation pathways of these compounds can be explained by the acid-catalyzed cyclization of citral and subsequent acid-catalyzed reactions such as isomerization, hydration, and dehydration (*1*−*3*). The other group was the oxidation products from citral, such as *p*-cresol (peak B), α,*p*-dimethylstyrene (peak C), *p*-methylacetophenone (peak E), *p*-cymen-8-ol (peak F), and 8-hydroperoxy-*p*-cymene (peak J). The formation pathways of these compounds would involve oxidation reactions as well as acid-catalyzed reactions (*1*, *3*, *4*, *7*). The effects of the theaflavin mixture (5 mg/L) on the formation of the products in each group are shown in **Figures 5** and **6**. Interestingly, the theaflavins strongly inhibited the formation of one of the acid-catalyzed reaction products, *p*-cymene. The other acid-catalyzed reaction products, *p*-menthadien-8-ols and *p*-menth-2-ene-1,8-diols, were slightly increased by the addition of the theaflavins. As for the oxidation products, the theaflavins inhibited the formation of *p*-cresol, *p*-methylacetophenone, and 8-hydroperoxy-*p*-cymene, whereas the formation of α,*p*-dimethylstyrene and *p*-cymen-8-ol was strongly promoted by the addition of the theaflavins.

Sensory Effect of Addition of Theaflavins. During the course of the experiments described above, we observed that

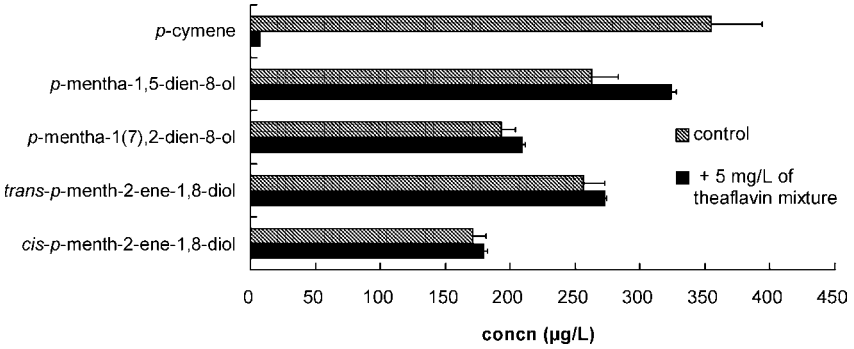


Figure 5. Effect of theaflavin mixture on the formation of acid-catalyzed reaction products from citral (10 mg/L) stored under acidic aqueous conditions (pH 3) at 40 °C for 2 weeks. Each value is expressed as the mean ± standard deviation of triplicate samples.

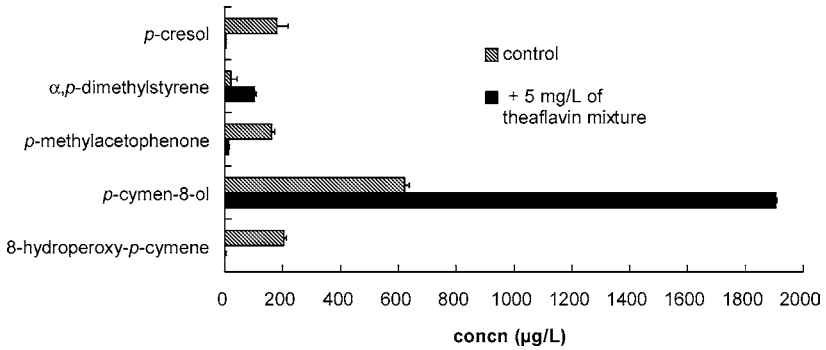


Figure 6. Effect of theaflavin mixture on the formation of oxidation products from citral (10 mg/L) stored under acidic aqueous conditions (pH 3) at 40 °C for 2 weeks. Each value is expressed as the mean ± standard deviation of triplicate samples.

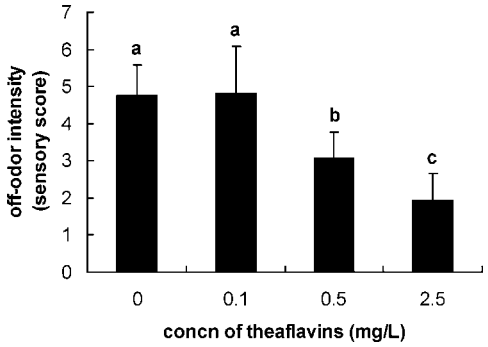


Figure 7. Off-odor intensity of stored citral solutions containing theaflavin mixture at different concentrations. Acidic aqueous solutions (pH 3) containing citral (10 mg/L) were stored at 40 °C for 2 weeks. Each value is expressed as the mean ± standard deviation ($n = 17$). Values with same letters are *not* significantly different ($p \geq 0.05$).

Table 3. Odor Thresholds of Possible Off-Odorants and Their Odor Activity Values (OAVs) in the Stored Citral Solutions

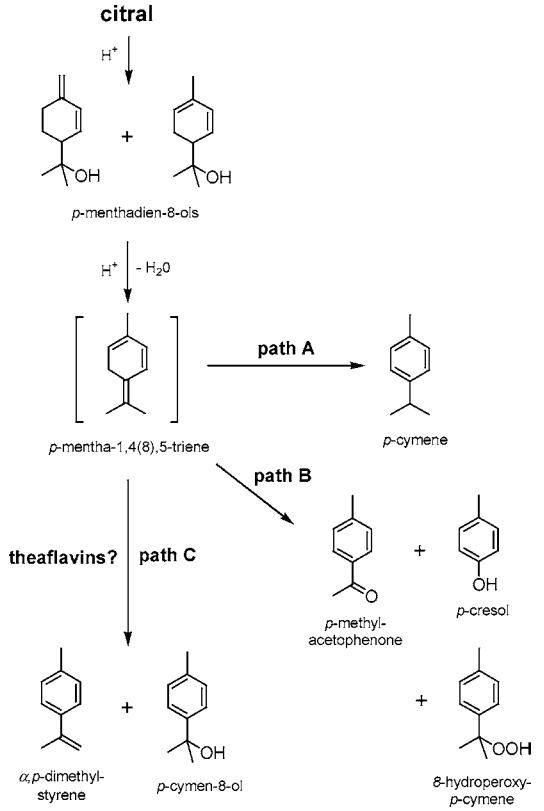
off-odorant	odor threshold in water[b] (μg/L)	OAVs in citral solutions[a] control	OAVs in citral solutions[a] + theaflavins
p-cymene	6.2–150[c–f]	2.4–55	0.05–1.1
p-cresol	2.7–55[g,h]	3.3–67	0.1–1.7
p-methylacetophenone	19–24[f,i,j]	6.7–8.5	0.6–0.8
8-hydroperoxy-*p*-cymene	nd[k]	nd	nd
α,*p*-dimethylstyrene	85[j]	0.3	1.2
p-cymen-8-ol	22500[l]	0.03	0.08

[a] OAVs were calculated by dividing the concentration of each off-odorant in the citral solutions (**Figures 5** and **6**) by its odor threshold. [b] Orthonasal detection. [c] Buttery et al. (*12*). [d] Ahmed et al. (*13*). [e] Pino et al. (*14*). [f] Boonbumrung et al. (*15*). [g] Buttery et al. (*16*). [h] Karagül-Yüceer et al. (*17*). [i] Buttery et al. (*18*). [j] Masanetz and Grosch (*19*, *20*). [k] Not determined. [l] Original data.

the undesirable odor qualities of the stored citral solutions, which can be described as "burnt", "phenolic", and "bitter-almond-like", were much improved by the addition of the theaflavins. To elucidate this ability of the theaflavins, the samples containing the theaflavin mixture at different concentrations were evaluated for their off-odor intensity (**Figure 7**). There was no significant difference between the samples with and without the theaflavins at a concentration of 0.1 mg/L. However, the addition of the theaflavins at concentrations of 0.5 and 2.5 mg/L significantly reduced the off-odor intensity of the samples, with a greater effect at the higher dose (2.5 mg/L). These results indicate that the off-odor-reducing ability of the theaflavin mixture is dose-dependent and noticeable enough at a concentration of 0.5 mg/L.

OAVs of Off-Odorants from Citral. To explain the sensory observation described above, the OAVs (*8*) were calculated for the possible off-odorants detected by the GC analysis (**Table 3**). The listed odorants were chosen because their concentrations were significantly changed by the addition of the theaflavins (**Figures 5** and **6**). The odor threshold values in water were taken from the literature (*12*−*20*) except for *p*-cymen-8-ol, for which the odor threshold in water was determined for the first time, showing an extremely higher value (22500 μg/L) than the values reported for the other off-odorants. The OAVs were calculated by dividing the concentration of each odorant in the citral solutions by its odor threshold (OAVs = concentration/odor threshold), which means that the odorants having OAVs >1 might contribute to the odor quality of the citral solution. Without the theaflavins, the OAVs of *p*-cymene, *p*-cresol, and *p*-methylacetophenone were much higher than the value of 1 (2.4−55, 3.3−67, and 6.7−8.5, respectively), suggesting their strong contribution to the odor quality of the citral solution. With the addition of the theaflavins, the OAVs of *p*-cymene, *p*-cresol, and *p*-methylacetophenone were significantly de-

Scheme 1. Previously Proposed Formation Pathways of Off-Odorants from Citral (*1*, *7*, *22*) and Possible Action of Theaflavins (Path A, Acid-Catalyzed Isomerization; Path B, Autoxidation; Path C, Dehydrogenation and Subsequent Hydration)



creased, so that even the highest calculated values were only 1.1, 1.7, and 0.8, respectively. This suggests that the odor intensity of these compounds has decreased to or below their detection levels. On the other hand, the OAV of α,*p*-dimethylstyrene just reached its detection level when the theaflavins were added (i.e., OAV = 1.2), and the OAV of *p*-cymen-8-ol was much lower than the value of 1 regardless of the addition of the theaflavins (0.03 and 0.08 without and with the theaflavins, respectively). On the basis of these data, it is apparent that the total OAVs of the listed off-odorants were significantly decreased by the addition of the theaflavins. The calculation of the OAVs with respect to the known odorants detected by GC and GC-MS might not provide sufficient information to explain the sensory quality of the samples, in comparison with more sophisticated methods, such as flavor reconstitution experiments using the odorants detected by GC−olfactometry (*21*). However, the data shown in **Table 3** agreed well with the sensory observation and therefore might be useful for explaining the off-odor-reducing ability of the theaflavins.

Mechanism of Off-Odor Inhibition by Theaflavins. The question remains as to why the distribution of odorants from citral can be changed by the addition of theaflavins, such as shown in **Figures 5** and **6**. A possible mechanism for these changes is as follows: On the basis of the literature and our previous study (*1*, *7*, *22*), the formation pathways of off-odorants from citral can be summarized as shown in **Scheme 1**. Citral degrades by acid-catalyzed reactions to form cyclization products such as *p*-menthadien-8-ols (*1*), which undergo further degradation via a possible intermediate, *p*-mentha-1,4(8),5-triene. As previously proposed (*7*), *p*-cymene can be formed by the acid-catalyzed isomerization of *p*-mentha-1,4(8),5-triene (path A), whereas the autoxidation of *p*-mentha-1,4(8),5-triene yields oxidation products, such as *p*-cresol, *p*-methylacetophenone, and 8-hydroperoxy-*p*-cymene (path B). In addition, the dehydrogenation of *p*-mentha-1,4(8),5-triene and subsequent hydration yield other oxidation products, α,*p*-dimethylstyrene and *p*-cymen-8-ol (path C) (*22*). The difference between the oxidative mechanisms in paths B and C is that the former involves direct combination with molecular oxygen, whereas the latter does not. In the present study, the theaflavins strongly inhibited the formation of *p*-cymene in path A as well as the oxidation products in path B, but promoted the formation of α,*p*-dimethylstyrene and *p*-cymen-8-ol in path C. This can be explained by the assumption that the theaflavins might be involved in the dehydrogenation of *p*-mentha-1,4(8),5-triene to α,*p*-dimethylstyrene in path C. It was reported that the enzymatic or chemical oxidation of theaflavin yielded a quinone compound named theanaphthoquinone, which has a 1,2-naphthoquinone ring derived from the benzotropolone moiety of theaflavin (*23*, *24*). Such theaflavin-derived quinones might act as dehydrogenating agents for the conversion of *p*-mentha-1,4(8),5-triene to α,*p*-dimethylstyrene, as reported for some of the synthetic quinones (*22*). On the basis of this assumption, further studies will be aimed at investigating the possible formation of quinone compounds resulting from the oxidative degradation of the theaflavins under our experimental conditions.

In conclusion, the results obtained in this study indicate that black tea theaflavins are potent inhibitors of the possible off-odorants *p*-cresol and *p*-methylacetophenone formed from citral under acidic aqueous conditions. In addition, the off-odor-reducing ability of the theaflavins was verified by the sensory evaluation and by calculation of the OAVs for the major degradation products from citral.

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