

# Antioxidant activity of plant extracts on the inhibition of citral off-odor formation

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Grape seed, pomegranate seed, green tea, and black tea extracts were used to inhibit the off-odor from citral degradation. A 0.1 M citrate buffer (pH 3), containing 100 ppm citral and 200 ppm gallic acid equivalent plant extract, was incubated at 40°C. The reaction mixtures were analyzed by high-performance liquid chromatography (HPLC) at days 0, 6, 10, 13, and 16 to monitor degradation of citral and formation of  $\alpha$ ,  $p$ -dimethylstyrene,  $p$ -cymene-8-ol, and  $p$ -methylacetophenone. The addition of plant phenolic extracts could not inhibit citral degradation, however, all four plant extracts significantly inhibited  $p$ -methylacetophenone formation. The samples, with the addition of plant extracts, exhibited higher concentrations of  $\alpha$ ,  $p$ -dimethylstyrene and  $p$ -cymene-8-ol than the control. This is presumed to be due to the oxygen-scavenging effect of plant extracts blocking the pathway from  $p$ -cymene-8-ol to  $p$ -methylacetophenone. Our results suggest that these plant extracts act as general antioxidants inhibiting the generation of  $p$ -methylacetophenone regardless of the types of water-soluble phenolic compounds existing in the plant extracts.

**Keywords:** Antioxidant / Citral degradation / Off-odor / Phenolic compounds

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## 1 Introduction

Citral, a monoterpene aldehyde, contributes to the strong, pleasant, lemon-like aroma in fresh lemon juice and lemon oil. Consisting of neral and geranial in the ratio of 2:3, citral is known to be unstable under acidic conditions [1–5], limiting its application in the beverage and fragrance industry. During degradation, citral undergoes a series of cyclization and oxidation processes. These reactions not only decrease the level of fresh lemon aroma, but also generate an off-odor. Under acidic conditions, the formation of off-odor compounds from citral is affected by pH, temperature, light, and the availability of oxygen [6–10]. Clark and Chamblee [11] summarized the reaction scheme of citral cyclization and secondary reaction in an acidic environment. The major intermediate compounds in the citral cyclization process were found to be  $p$ -mentha-1,5-dien-8-ol and  $p$ -mentha-1(7), 2-dien-8-ol. Further oxidation of these intermediate compounds resulted in the generation of

$p$ -cymene-8-ol. This alcohol undergoes a dehydration process and transforms to  $\alpha$ ,  $p$ -dimethylstyrene. Kimura *et al.* [5] reported that  $\alpha$ ,  $p$ -dimethylstyrene and  $p$ -cymene were responsible for the off-odor of deteriorated lemon. In an oxygen environment, the end products of citral degradation consist of 80%  $\alpha$ ,  $p$ -dimethylstyrene and 8% cymene. The ratio changes to 17% of  $\alpha$ ,  $p$ -dimethylstyrene and 81% of cymene in a nitrogen environment [6]. More recently, Schieberle *et al.* [12] indicated that  $p$ -methylacetophenone and  $p$ -cresol were more responsible for the undesirable flavor in citral degradation than  $\alpha$ ,  $p$ -dimethylstyrene, based on aroma extraction dilution analysis (AEDA). Kimura *et al.* [5] attempted to use 2,6-di-*tert*-butyl- $p$ -cresol (BHT), 3-*tert*-butyl-4-hydroxyanisoles (BHA),  $n$ -propyl gallate,  $\alpha$ -tocopherol, nordihydroguaiaretic acid, and  $n$ -tritiacontan-16,18-dione to inhibit the deterioration of citral. These compounds were found to have no effect on citral degradation. In contrast, Peacock and Kuneman [7] found isoascorbic acid to be able to inhibit the formation of  $p$ -cymene-8-ol and its dehydration product,  $\alpha$ ,  $p$ -dimethylstyrene, in a carbonated beverage system containing citral.

Phenolic compounds from plants are well-known for their oxygen-scavenging activity and are well-recognized to be effective as antioxidants [13–16]. The objective of this study was to use commercially available plant extracts to inhibit the generation of off-odor compounds resulting

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**Abbreviations:** rBHA, 3-*tert*-butyl-4-hydroxyanisole; BHT, 2,6-di-*tert*-butyl- $p$ -cresol

from citral degradation. Four commercially available plant extracts (green tea, black tea, grape seed, and pomegranate seed extracts) were selected based on their high phenolic content and were used as antioxidants in this research.

## 2 Materials and methods

### 2.1 Chemicals and plant extracts

Citral, *p*-methylacetophenone, and sodium carbonate were purchased from Aldrich (St. Louis, MO, USA). Gallic acid, phosphoric acid 85%, HPLC-grade acetonitrile, isopropanol, and methylene chloride were obtained from Fisher Scientific (Springfield, NJ, USA). Folin-Ciocalteu phenol reagent was from Sigma (St. Louis, MO, USA). *p*-Cymene-8-ol and  $\alpha$ , *p*-dimethylstyrene were gifts from Ogawa (Chiba, Japan). Acidic buffer solution (pH 3) was prepared by mixing 0.1 M citric acid with 0.2 M sodium phosphate (dibasic). Paraffin mix was from Supelco (Bellefonte, PA, USA). Black tea, green tea, pomegranate seed, and grape seed extracts were selected as natural phenolic antioxidants and were gifts from Bannerbio International (Shenzhen, P. R. China). The plant extracts were identified by HPLC methods with reference standards.

### 2.2 Total phenolic content

The total phenolic content of each plant extract was determined photometrically at 725 nm (HP 8453 spectrophotometer; Hewlett-Packard, Waldbronn, Germany). The plant extract was dissolved in ethanol and reacted with Folin-Ciocalteu phenol reagents according to the procedure described by Singleton and Rossi [17]. First, 40 mg of plant extract was dissolved in 40% ethanol and sonicated for 30 min. After cooling to room temperature, the solution was filled to the full volume of 100 mL with extraction solvent. The extract was filtered and the clear solution was used for phenolics quantification. After diluting 10-fold with distilled water, 0.5 mL of solution was mixed with 4.5 mL distilled water, 0.2 mL Folin-Ciocalteu reagent and 0.5 mL saturated sodium carbonate solution in a test tube. The mixture was vortexed and 4.3 mL of distilled water was added to the test tube. Samples were allowed to stand for 1 h at room temperature and UV absorption of each mixture was measured at 725 nm. Gallic acid was used as calibration standard.

### 2.3 Model solutions

One mL of 10,000 ppm citral in ethanol was mixed with 10 mL plant extract in ethanol that contained 2000 ppm phenolics. Ethanol (9 mL) and 80 mL acidic buffer (pH 3) were added to the reaction mixture to bring the final citral

and plant extract concentrations to 100 ppm and 200 ppm, respectively. The reaction mixtures were kept in airtight amber glass bottles and incubated in a 40°C water bath. The generation of *p*-methylacetophenone, *p*-cymene-8-ol, and  $\alpha$ , *p*-dimethylstyrene were monitored on days 6, 10, 13, and 16 by HPLC.

### 2.4 Extraction of volatile compounds for GC and GC-MS analysis

After incubation in a 40°C water bath for 16 days, 50 mL reaction mixture was extracted with methylene chloride (10 mL  $\times$  3). Undecane was added as internal standard. The extract was dried over anhydrous sodium sulfate and concentrated to 1000  $\mu$ L under reduced pressure. The extract was further concentrated to 300  $\mu$ L under nitrogen.

### 2.5 HPLC analysis

HPLC analyses were performed on a Waters 2695 separation modular system equipped with Waters 2996 photodiode array detector with a Millennium data system (Milford, MA, USA). Solvent system: mobile phase A, 0.1% phosphoric acid; mobile phase B, acetonitrile; mobile phase C, isopropanol. To identify different plant materials, 50 mg of plant extract was dissolved in 25 mL 70% methanol solution. Ten  $\mu$ L sample was injected for HPLC analysis. To analyze the off-flavor compounds, 30  $\mu$ L reaction solution was directly injected to HPLC. The columns and chromatographic conditions used to analyze plant extracts and citral model solutions were as follows:

#### 2.5.1 Identification of black tea extract

A Prodigy ODS3 analytical column (Phenomenex, Torrance, CA, USA), 150  $\times$  3.20 mm (5  $\mu$ m particle size, 00F-4097-R0) was used. The linear gradient of eluents A (0.1% phosphoric acid) and B (acetonitrile) was performed as follows: 90% to 80% A for the first 12 min, hold at 80% A for 8 min. From 20 to 28 min, 80% A gradient to 70% A, from 28 min to 40 min, 70% A gradient to 60% A. The flow rate was 1.1 mL/min and the detection wavelength was set at 272 nm. Theaflavin (TF), theaflavin-3-gallate (TF3G), theaflavin-3'-gallate (TF3'G), and theaflavin-digallate (TFDG) were identified based on the comparison of retention time and UV absorption curve with the standards.

#### 2.5.2 Identification of pomegranate seed extract

A Phenomenex Prodigy ODS3 analytical column, 150  $\times$  3.20 mm (5  $\mu$ m particle size, 00F-4097-R0) was used. The linear gradient of eluents A (0.1% phosphoric acid) and B (acetonitrile) was performed as follows: for the first 20 min, the percentage of A changed from 95% to 85%, from

20 min to 35 min the gradient changed from 85% to 80% A. The flow rate of the mobile phase was 1.0 mL/min and the detection wavelength was set at 254 nm. Ellagic acid was identified based on the comparison of retention time and UV absorption curve with the standard.

### 2.5.3 Identification of grape seed extract

A Luna C18(2) analytical column (Phenomenex), 250 × 4.60 mm (5 µm particle size, 00G-4252-E0) was used. The linear gradient of eluents A (0.1% phosphoric acid) and B (acetonitrile) was performed as follows: for the first 15 min the percentage of A changed from 100% to 94%; from 15 min to 30 min the gradient changed from 94% to 88% A; from 30 min to 50 min the gradient changed from 88% to 82% A; from 50 to 70 min the gradient changed from 82% to 75% A; from 70 to 80 min the gradient changed from 75 to 50% A, and was held for 5 more min at 50% A. The flow rate of the mobile phase was 1.0 mL/min and the detection wavelength was 280 nm. Catechin and epicatechin were identified based on comparison of the retention time and UV absorption curve with the standards.

### 2.5.4 Identification of green tea extract

A Luna C18(2) analytical column (Phenomenex), 250 × 4.60 mm (5 µm particle size, 00G-4252-E0) was used. The column temperature was set at 30°C and the mobile phase included water (containing 0.1% phosphoric acid, solvent A), acetonitrile (solvent B), isopropanol (solvent C) in a gradient system. The total running time was 40 min. 0 min, 92% solvent A, 8%; 16 min, 86% A, 12% B, and 2% C; 25 min, 78% A, 20% B, and 2% C; 30 min, 60% A, 38% B, and 2% C; 31 min, 92% A and 8% B; and 40 min, 92% A and 8% B. The flow rate was 1.0 mL/min, the injection volume 10 µL, and the detection wavelength was set at 280 nm. Epigallocatechin (EGC), catechin (C), epicatechin (EC), epigallocatechin gallate (EGCG), gallic acid (GA), gallic acid gallate (GCG), epicatechin gallate (ECG), and catechin gallate (CG) were identified based on the comparison of retention time and UV absorption curve with the standards.

### 2.5.5 Analysis of off-flavor compounds by HPLC

A prepacked 150 × 3.20 mm (5 µm particle size, 00F-4097-R0) Prodigy ODS3 analytical column (Phenomenex) was used. Thirty µL sample was kept at 4°C, injected into HPLC, and analyzed by a gradient mobile phase system consisting of eluents A (water with 1% phosphoric acid) and B (acetonitrile). The linear gradient of eluents A and B was performed as follows: 85% A for the first 5 min, 85% to 15% A from 5 to 50 min. The flow rate was 1.0 mL/min. *p*-Methylacetophenone, *p*-cymene-8-ol, and  $\alpha$ , *p*-dimethylstyrene were identified based on the comparison of retention time and UV absorption curve with the reference standards.

## 2.6 GC-mass analysis of degradation products

An Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass detector and HP-5 MS 5% phenylmethyl siloxane column (30.0 m × 250 µm × 0.25 µm) was used. Five µL sample was injected with the split ratio of 1:10. The injection port was kept at 220°C. The gas flow was controlled as follows: hydrogen flow at 40.0 mL/min, air flow at 450 mL/min, and nitrogen flow 45.0 mL/min. The oven temperature was increased from 60 to 110°C at 1°C/min for the first 50 min, then to 210°C at 2°C/min, and held at 210°C for 5 min. The mass detector was operated at the electronic ionization mode. The ionization voltage was held at 70 eV and the ion temperature was at 280°C.

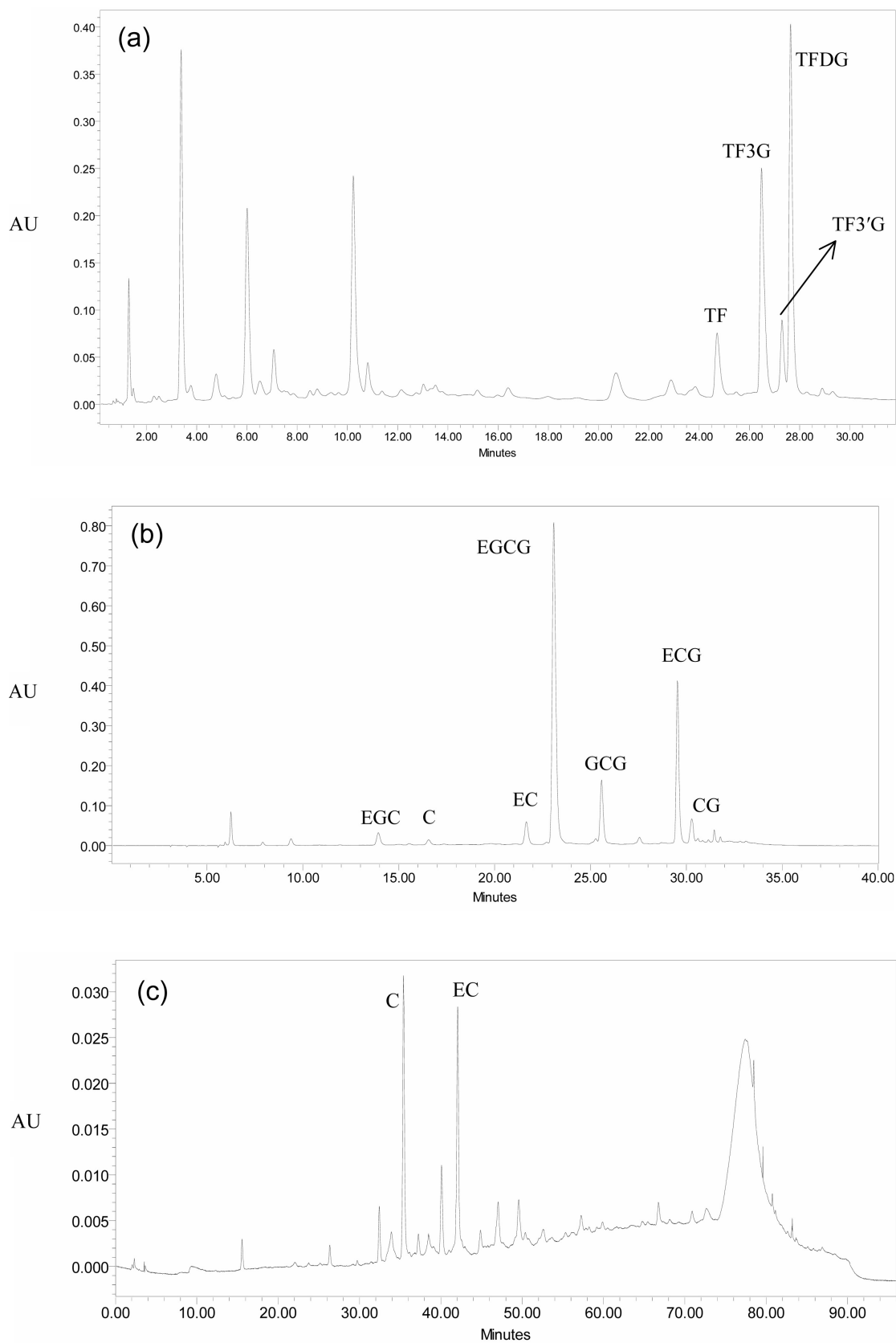
## 2.7 GC analysis

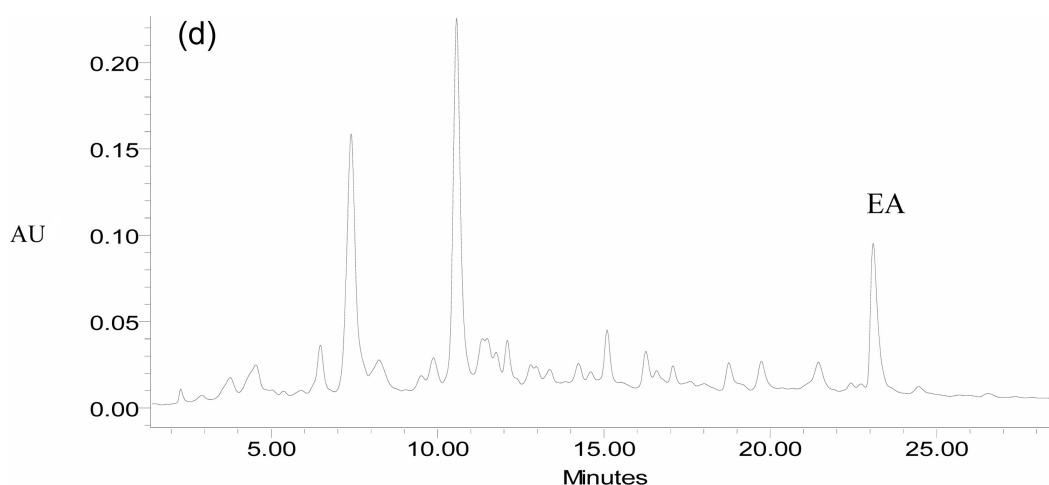
An Agilent 6890 gas chromatograph equipped with a flame ionization detector and HP-5 MS 5% phenylmethyl siloxane column (30.0 m × 250 µm × 0.25 µm) was used. One µL sample was injected with a split ratio of 1:200. The inlet port and the detector were kept at 220 and 250°C, respectively. The gas flow was controlled as follows: hydrogen flow at 40.0 mL/min, air flow at 450 mL/min, and nitrogen flow 45.0 mL/min. The oven temperature was increased from 60 to 110°C at 1°C/min for the first 50 min, then to 210°C at 2°C/min, and held at 210°C for 5 min. Citral degradation products were quantified by computing the peak area *versus* the peak area of the internal standard (undecane). Retention indices were determined according to Halang *et al.* [18].

## 3 Results and discussion

### 3.1 HPLC analysis of plant extracts

Phenolic compounds are well-known for their antioxidant activities. In this research we evaluated the ability of black tea, green tea, grape seed, and pomegranate seed phenols to preserve citral, an important flavor compound. Before application of these plant extracts to preserve flavor, the phenols were identified by HPLC analysis using in-house developed methods, and analyses of targeted marker compounds in each plant were performed. In black tea, four marker compounds, namely theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin-3,3'-digallate, were identified. In green tea extract, seven major tea catechins were found to be present in significant concentrations while the caffeine content was very low. Using HPLC, the different proanthocyanidins were examined in grape seed extract, and proanthocyanidin polymers were found to be the major components. In pomegranate seed extract, the major marker compound ellagic acid was observed. The





**Figure 1.** (a) HPLC chromatogram of black tea extract at 272 nm. Peak at 24.83 min, theaflavin (TF); at 26.58 min, theaflavin-3-gallate (TF3G); at 27.37 min, theaflavin-3'-gallate (TF3'G); and at 27.71 min, theaflavin-digallate (TFDG). (b) HPLC chromatogram of green tea extract at 272 nm. Peak at 13.93 min, epigallocatechin (EGC); at 16.56 min, catechin (C); at 21.66 min, epicatechin (EG); at 23.09 min, epigallocatechin gallate (EGCG); at 25.57 min, gallic catechin gallate (GCG); at 29.53 min, epicatechin gallate (ECG); and at 30.28 min, catechin gallate (CG). (c) HPLC chromatogram of grape seed extract at 272 nm. Peak at 35.42 min, catechin (C); peak at 42.07 min, epicatechin (EC). The large peak at 77.98 min consists of proanthocyanidin polymers. (d) HPLC chromatogram of pomegranate seed at absorbance 272 nm. Peak at 23.10 min, ellagic acid (EA).

HPLC chromatograms of these four plant extracts are shown in Figs. 1 a–d.

### 3.2 Phenolic contents of the plant extracts

The phenolic contents of each plant extract were quantified based on gallic acid as the standard, using a colorimetric method [17]. All plant extracts were found to contain a high amount of phenols with green tea extract having the highest phenolic content of the four plant extracts (Table 1). These data were used to prepare a 200 ppm gallic acid equivalent (GAE) of phenolics, in ethanol, from each plant extract.

**Table 1.** Phenolic content (gallic acid equivalent) of selected plant extracts

Extract	Phenolic content (%)
Black tea	61.51 ± 3.76
Green tea	81.63 ± 2.15
Grape seed	74.23 ± 2.32
Pomegranate seed	65.50 ± 1.97

### 3.3 HPLC analysis of the formation of off-flavor in model reaction

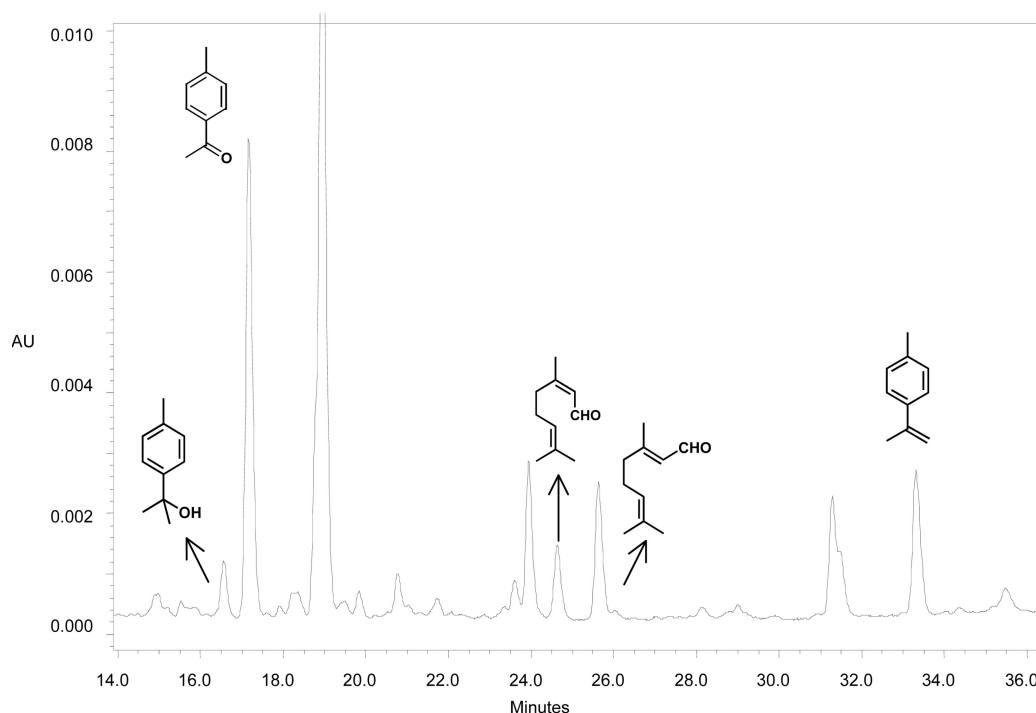
The generation of *p*-methylacetophenone, *p*-cymene-8-ol and  $\alpha$ , *p*-dimethylstyrene in the model reaction was monitored at day 6, 10, 13, and 16 by HPLC. One representative HPLC chromatogram of 100 ppm citral solution (pH 3) without the addition of the plant extract, and kept at 40°C for 16 days is shown in Fig. 2. After 16 days incubation, the

amount of neral and geranial existing in the acidic solution was less than 2% of the original amount added into the solution. Thus, the addition of these plant extracts neither inhibited nor slowed down citral degradation (Figs. 3 a and b). In contrast, all four plants extracts significantly inhibited the formation of *p*-methylacetophenone (Fig. 4 a), while samples with the addition of plant extracts exhibited higher concentrations of  $\alpha$ , *p*-dimethylstyrene and *p*-cymene-8-ol than the control (Figs. 4 b and c). We presume this to be due to an oxygen-scavenging effect blocking the pathway from *p*-cymene-8-ol to *p*-methylacetophenone (for possible pathway see Fig. 5).

The concentration of *p*-cymene-8-ol increased rapidly on day 9 while *p*-methylacetophenone and  $\alpha$ , *p*-dimethylstyrene increased gradually throughout the 16 days. This may be due to the detection limit of the photodiode array detector < 254 nm. *p*-Cymene-8-ol exhibited relatively lower UV absorbance than *p*-methylacetophenone and  $\alpha$ , *p*-dimethylstyrene at < 254 nm wavelength. These results suggest that the plant extracts do act as antioxidants by inhibiting the generation of *p*-methylacetophenone, regardless of the specific presence of water-soluble phenolic compounds from the different plant extracts.

### 3.4 GC-MS analysis

GC-MS was used to identify the degradation products of citral. GC-Mass analysis identified 14 compounds based on the retention index from the literature [7], Wiley mass spec-



**Figure 2.** HPLC chromatogram of citral solution stored at 40°C for 16 days under acidic condition. Peak at 16.50 min, *p*-cymene-8-ol; at 17.21 min, *p*-methylacetophenone; at 24.42 min, neral; at 25.43 min; geranial; at 33.35 min,  $\alpha$ , *p*-dimethylstyrene. Chromatograms were shown at 254 nm.

tral database (Agilent Technologies), or the mass spectrum and the retention index of the authentic compounds. The identification method used for each compound is listed in Table 2.

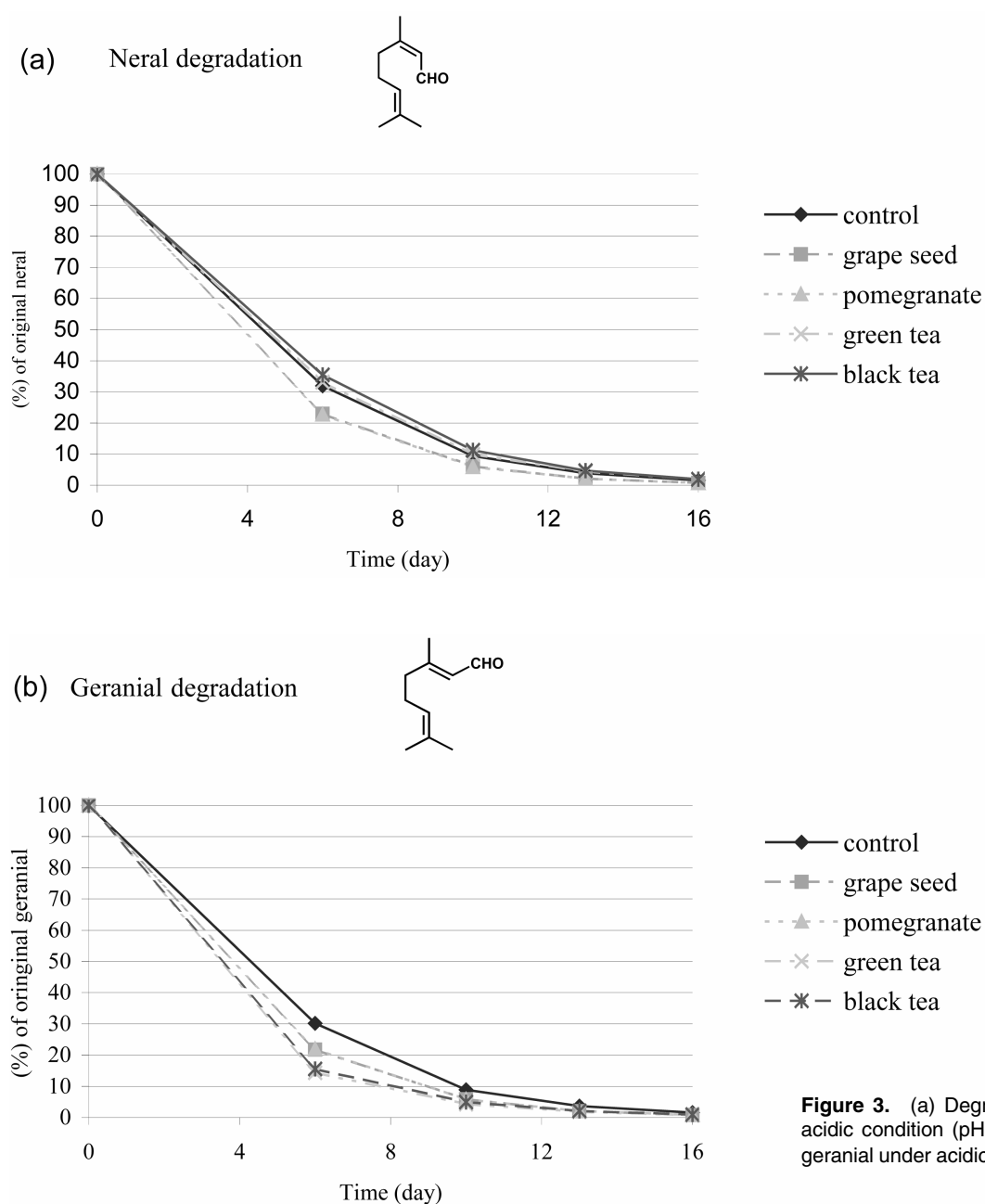
### 3.5 GC analysis

GC was used to analyze for the content of different degradation products, and the concentration of some compounds generated during citral degradation (Tables 3 and 4). Comparing the data obtained from both GC and HPLC analyses, the same trend could be observed: addition of plant extracts significantly inhibited the formation of *p*-methylacetophenone (Table 3) and led to increased concentrations of  $\alpha$ , *p*-dimethylstyrene and *p*-cymene-8-ol (Table 4). Samples with the addition of black tea extract exhibited the highest concentration of  $\alpha$ , *p*-dimethylstyrene and *p*-cymene-8-ol in both HPLC and GC analyses. However, the concentration of citral degradation products quantified by HPLC are higher than from GC analysis. This may be due to either loss during GC sample preparation or from further degradation of compounds during GC analysis. Relative to GC analysis, HPLC analysis offers a convenient way to monitor the citral degradation and the formation of off-odor compound formation.

**Table 2.** Compounds formed from citral degradation

Compd. No.	Compound	ID Method <sup>a)</sup>
1	2,3-Dehydro-1,3-cineole	B
2	<i>p</i> -Cymene	A
3	<i>p</i> -Cresol	A
4	$\alpha$ , <i>p</i> -Dimethylstyrene	A
5	<i>p</i> -Metha-1,5-diene-8-ol	B
6	<i>p</i> -Methylacetophenone	A
7	<i>p</i> -Cymene-8-ol	A
8	$\alpha$ -Terpineol	A
9	<i>p</i> -Metha-1(7),2-diene-8-ol	B
10	(2,5)-2-Formylmethyl-2-methyl-5-hydroxy-1-methylethyl)-tetrahydrofuran isomer	C
11	(2,5)-2-Formylmethyl-2-methyl-5-hydroxy-1-methylethyl)-tetrahydrofuran isomer	C
12	<i>trans-p</i> -menth-2-ene-1,8-diol	B
13	<i>cis-p</i> -menth-2-ene-1,8-diol	B
14	8-Hydroperoxy- <i>p</i> -cymene	A

a) Compounds were identified on the basis of the following criteria: A, mass spectrum and retention index agree with those of authentic compounds provided by Ogawa Company (Chiba, Japan); B, mass spectrum agrees with that of Wiley mass spectral database and retention index (RI) agrees with literature value; C, mass spectrum agree with literature spectrum [17]. Compounds identified in the categories B and C can only be considered as “tentatively identified”.



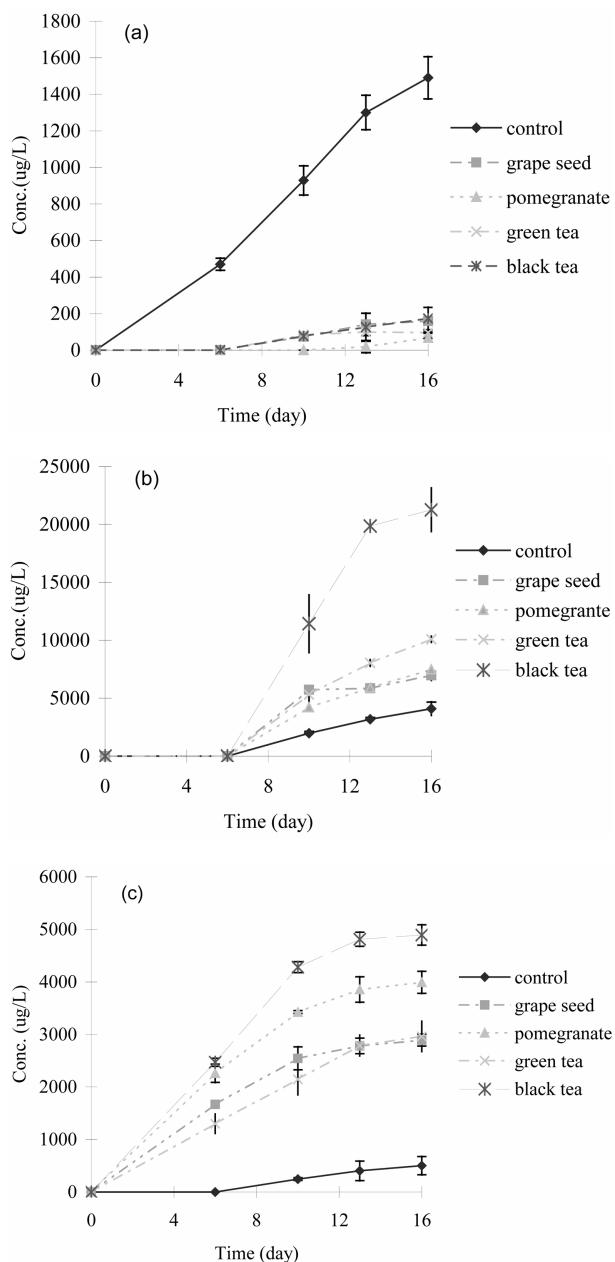
**Figure 3.** (a) Degradation of neral under acidic condition (pH 3). (b) Degradation of geranial under acidic condition (pH 3).

**Table 3.** Inhibition of off-odor from citral degradation by plant extracts

Plant extract	Formation of compounds ( $\mu\text{g/L}$ )			
	<i>p</i> -Cymene	<i>p</i> -Cresol	<i>p</i> -Methyl acetophenone	8-Hydroperoxy- <i>p</i> -cymene
Control	918.3 $\pm$ 52.1	936.5 $\pm$ 34.1	806.9 $\pm$ 43.7	1109.2 $\pm$ 99.2
Grape seed	95.9 $\pm$ 11.2	296.1 $\pm$ 5.8	*	27.2 $\pm$ 0.5
Pomegranate seed	100.7 $\pm$ 13.3	*	*	*
Green tea	121.9 $\pm$ 10.9	*	*	*
Black tea	*	*	*	*

\* Compound was detected in trace amount.

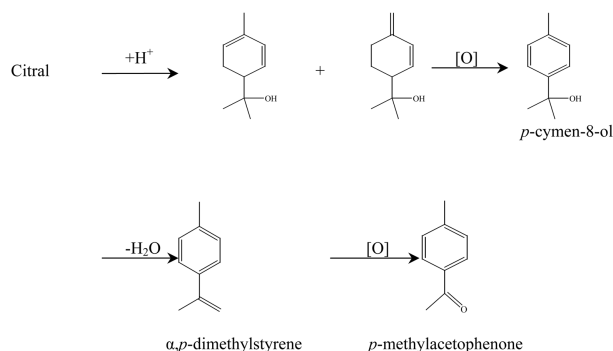
The concentration was quantified by GC analysis.



**Figure 4.** Effect of plant extracts on the formation of (a) *p*-methylacetophenone, (b) *p*-cymene-8-ol, and (c)  $\alpha$ , *p*-dimethylstyrene.

The standard compound for each degradation product is needed with either analysis to quantify the compound.

Kimura *et al.* [5] unsuccessfully tried to use BHT, BHA, *n*-propyl gallate,  $\alpha$ -tocopherol, nordihydroguaiaretic acid, and *n*-tritracontan-16,18-dione to inhibit the generation of off-odor compounds (*p*-cymene-8-ol, *p*-cymene, and  $\alpha$ , *p*-dimethylstyrene) from citral deterioration at 38 °C. In contrast, based upon our GC data, the formation of *p*-cymene



**Figure 5.** Proposed reaction scheme of acid-catalyzed breakdown of citral [12].

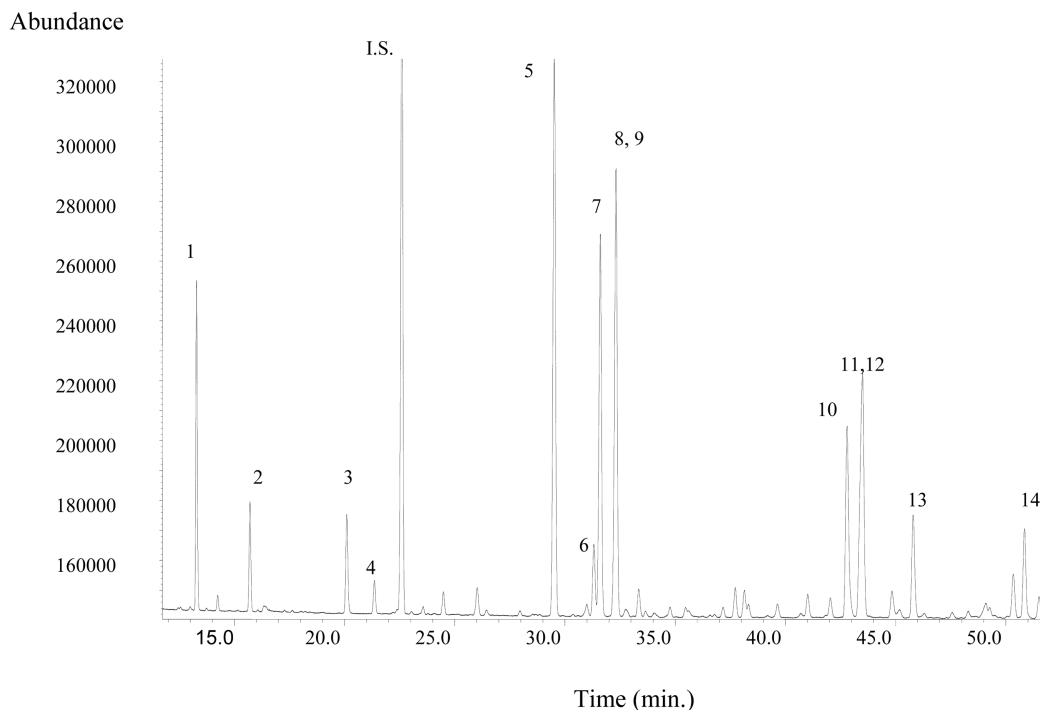
**Table 4.** Promotion of  $\alpha$ , *p*-dimethylstyrene and *p*-cymene-8-ol formed from citral degradation by plant extracts

Plant extract	Formation of compounds ( $\mu\text{g/L}$ )	
	$\alpha$ , <i>p</i> -Dimethylstyrene	<i>p</i> -Cymene-8-ol
Control	230.8 $\pm$ 29.7	4547.5 $\pm$ 327.8
Grape seed	1027.1 $\pm$ 33.4	8056.6 $\pm$ 186.5
Pomegranate seed	1384.3 $\pm$ 43.7	9177.1 $\pm$ 198.8
Green tea	982.1 $\pm$ 80.8	7762.7 $\pm$ 165.8
Black tea	1580.8 $\pm$ 54.6	10175.1 $\pm$ 337.4

The concentrations were quantified by GC analysis.

was inhibited by the addition of plant extracts while the formation of *p*-cymene-8-ol and  $\alpha$ , *p*-dimethylstyrene were induced. The ratio of the antioxidant Kimura *et al.* [5] added to the citral solution was from 1 : 50 to 1 : 20 (antioxidant : citral w/w). In our research, the amount of plant extract added into the citral solution was equal to 200 ppm gallic acid resulting in a ratio of antioxidant to citral of 2 : 1. Thus, the ratio of antioxidant to citral we used was much higher than the ratio Kimura *et al.* [5] used. In addition, the acidic condition of their citral solution was achieved by adding citral to 7% citric acid solution, thus the pH of their model solution was more acidic than the pH used in our study (pH 3). According to Diehl [19], the oxygen potential of oxygen in water is decreased with the increase in pH. Thus, the failure of Kimura [5] to inhibit off-odor compounds from citral degradation could be related to the harsh conditions they used. Ueno *et al.* [20] successfully inhibited the formation of *p*-methylacetophenone from citral degradation using pure tea catechins. They also reported that *p*-cresol is the formation intermediate from 8-hydroperoxy-*p*-cymene. Finally, we only tested the effect of plant extracts on citral degradation at pH 3. Further investigation of plant extracts on citral degradation across a pH gradient is now needed to ascertain whether the citral degradation can be controlled.





**Figure 6.** Representative gas chromatogram of citral under acidic condition (pH 3) following storage at 40°C for 16 days. Numbers correspond to those in Table 2. Undecane was used as internal standard (I.S.).

#### 4 Concluding remarks

Based upon our data, we can conclude that the addition of phenolic compounds from plants can inhibit off-odor formation from citral degradation at pH 3 regardless of the types of phenolic compounds used from the four different plant extracts. The inhibition of off-odor generation from citral degradation by antioxidant appears to depend on the concentration of the antioxidant. If moderate amounts of antioxidants were used, the off-odor from citral deterioration can be greatly reduced. Addition of plant extracts significantly inhibits the formation of *p*-cymene (2), *p*-cresol (3), *p*-methylacetophenone (6), and 8-hydroperoxy-*p*-cymene (14) (Table 3). According to Schieberle and Grosch [21], *p*-methylacetophenone (2.7–10.8 ng/L air) and *p*-cresol (0.3–1.0 ng/L air) have significantly lower odor threshold than  $\alpha$ , *p*-dimethylstyrene (665–2660 ng/L air). Although the addition of plant extracts promotes the generation of  $\alpha$ , *p*-dimethylstyrene, as long as the formation of *p*-methylacetophenone and *p*-cresol is inhibited, the off-odor of citral under acidic conditions can be reduced.

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