

Methods for Thermal Stability Enhancement of Leaf Essential Oils and Their Main Constituents from Indigenous Cinnamon (*Cinnamomum osmophloeum*)

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ABSTRACT: The thermal stability of leaf essential oils from various *Cinnamomum osmophloeum* and their constituents was investigated for the first time. The results indicated that *trans*-cinnamaldehyde (Cin) content in eugenol-free essential oil from *C. osmophloeum* was affected by high temperatures. The retention of Cin (RC) decreased to 17.4% after the essential oil was incubated for 8 h at 100 °C. In contrast, essential oils containing eugenol showed greater thermal stability. Seven kinds of antioxidants were added to Cin to improve its thermal stability. Among them, eugenol endowed Cin with the best thermal stability. We also investigated the influence of various amounts of eugenol on the thermal stability of both essential oil and Cin. Both essential oil and Cin showed excellent thermal stability when 0.62 and 2.60% (v/v) eugenol were added. In short, the thermal stability of essential oil and Cin could be effectively improved by adding appropriate amounts of eugenol.

KEYWORDS: Antioxidant, *trans*-cinnamaldehyde, *Cinnamomum osmophloeum*, eugenol, leaf essential oil, thermal stability

INTRODUCTION

Essential oil is mainly composed of terpenoids and aromatic compounds that readily deteriorate under high temperature, strong light, and reactive oxygen species. Some studies have confirmed that constituents in essential oil might be affected by environmental factors during storage.¹ In addition, safety and biological activities of essential oils also changed after storage.^{2,3} Antioxidant activities and the shelf life of each essential oil vary, owing to their different compositions. Essential oils that contain eugenol, thymol, carvacrol, and guaiacol showed great antioxidant activity and could be preserved longer.^{1,4}

The genus *Cinnamomum* comprises over 250 aromatic evergreen trees and shrubs, which are indigenous to Asia and Australia.⁵ Cinnamon (*Cinnamomum cassia* Nees) is a common spice with sweet, spicy, and special flavor. It has been widely used in bakeries, drinks, desserts, and cuisines. The main constituent of essential oil from cinnamon bark is *trans*-cinnamaldehyde (Cin). Some reports claimed that leaf essential oil of indigenous cinnamon (*Cinnamomum osmophloeum* Kanehira) contained a large amount of Cin. Therefore, *C. osmophloeum* has been used as a spice substitute for *C. cassia*.^{6–8} It was primarily used in the fragrance and flavor industries for imparting a cinnamon flavor and/or fragrance to various types of foods, medical products, beverages, and perfumes.⁹ This plant species was not only important as a spice, but in east Asia, leaf essential oil and Cin also showed various bioactivities, including antibacterial,^{8,10} antioxidant,¹¹ anticancer,⁹ antifungal,^{5,12,13} antitermitic,^{14,15} mosquito larvicidal,^{16,17} antimildew,¹⁸ antipathogenic,^{19,20} anti-red imported fire ants,²¹ antihyperuricemic,²² anti-inflammatory,^{23,24} and antidyslipi-

demie²⁵ activities. However, domestic producers claimed that the thermal stability of essential oils from *C. osmophloeum* was poor. As a result, the quality of related products of leaf essential oil after manufacturing processes was not as good.

To extend the shelf life of leaf essential oil of *C. osmophloeum*, this study investigated for the first time the thermal stability of leaf essential oil and its major components. The appropriate antioxidants and their dosages were optimized to establish a method for protecting leaf essential oil and Cin from thermal oxidation.

MATERIALS AND METHODS

Plant Materials. Fresh mature leaves of three *C. osmophloeum* provenances (COA, CO11701; COB, CO11401; and COC, CO11301) were collected in January 2008 from Fushan Experimental Forest located in Yilan, Taiwan, and those of two *C. osmophloeum* provenances (COD, CO1713; and COE, CO0302) were collected in October 2007 and August 2008, respectively, from Lienhuachih Research Center in Nantou, Taiwan. The species were identified by Sheng-You Lu and Yen-Ray Hsui who work at the Taiwan Forestry Research Institute, and voucher specimens were deposited at the Laboratory of Wood Chemistry, School of Forestry and Resource Conservation, National Taiwan University.

Chemicals. Ethyl acetate was purchased from ECHO Chemical Co., Ltd. (Taiwan). The following compounds were employed as standards in gas chromatography–mass spectrometry (GC–MS)

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Table 1. Constituents and Contents (%) of Leaf Essential Oils from Five Provenances of *C. osmophloeum*

KI ^a	compound	specimen				
		A ^b	B	C	D	E
939	α -pinene	c	0.10	0.57	0.38	c
955	camphene	c	0.09	0.32	0.33	c
967	benzaldehyde	3.47	1.56	3.63	5.91	0.76
982	β -pinene	c	0.04	0.22	c	c
1028	<i>p</i> -cymene	0.63	c	c	c	c
1033	limonene	c	0.04	0.24	0.14	c
1036	1,8-cineol	c	c	c	0.33	c
1099	linalool	c	c	c	c	14.38
1165	benzenepropanal	4.47	0.96	2.97	3.88	0.71
1196	α -terpineol	c	c	0.10	c	0.17
1198	4-allylanisole	0.94	0.26	0.35	0.94	0.25
1221	<i>cis</i> -cinnamaldehyde	0.64	0.72	0.61	0.34	0.81
1274	<i>trans</i> -cinnamaldehyde	88.74	95.25	87.25	81.46	68.44
1285	L-bornyl acetate	0.27	0.44	0.95	1.27	c
1285	<i>trans</i> -anethole	c	c	c	c	0.75
1352	eugenol	0.43	c	0.65	0.28	1.26
1376	(-)- α -copaene	c	0.04	0.23	0.21	c
1419	<i>trans</i> - β -caryophyllene	0.13	0.17	0.55	0.69	0.58
1445	<i>trans</i> -cinnamyl acetate	0.21	c	0.74	3.31	10.88
1456	α -humulene	c	c	0.06	c	c
1516	δ -cadinene	c	0.07	0.29	c	0.32
1519	spathulenol	c	c	c	0.33	c
1580	caryophyllene oxide	c	0.17	0.27	0.18	0.11
1611	cedrol	c	0.02	c	c	c
1646	δ -cadinol	c	c	c	c	0.25
1647	T-muurolol	c	0.03	c	c	c
1659	α -cadinol	0.06	0.04	c	c	0.29
total		99.99	100.00	100.00	99.98	99.96

^aKI = Kovats indices relative to *n*-alkanes (C₉–C₂₁) on a DB-5 ms column. ^bA–E = *C. osmophloeum* A (CO11701), *C. osmophloeum* B (CO11401), and *C. osmophloeum* C (CO11301) were collected from the Fushan Experimental Forest, and *C. osmophloeum* D (CO1713) and *C. osmophloeum* E (CO0302) were collected from the Lienhuachih Research Center. ^cNot detected.

analyses. Benzaldehyde (98%), benzenepropanal (97%), L-bornyl acetate (95%), caryophyllene oxide (95%), *trans*-cinnamyl acetate (99%), *p*-cymene (99%), eugenol (99%), (+)-limonene (97%), (-)-limonene (92%), linalool (97%), α -pinene (97%), β -pinene (98%), α -terpineol (97%), thymol (99%), and α -tocopherol (99%) were all purchased from Acros (Morris Plains, NJ). *trans*-Anethole (95%) and camphene (95%) were both purchased from ICN (Costa Mesa, CA). *trans*- β -Caryophyllene (95%), 1,8-cineol (95%), octyl gallate (98%), propyl gallate (98%), and *n*-tetradecane (99%) were all purchased from TCI (Tokyo Chemical Industry Co., Ltd., Japan). Cin (98%) was purchased from Alfa Aesar (U.K.), and (-)- α -copaene (90%) was purchased from Fluka (Germany). Both butylated hydroxytoluene (99%) and carvacrol (98%) were purchased from Sigma-Aldrich (St. Louis, MO).

Distillation of Essential Oils. Fresh mature leaves of *C. osmophloeum* were air-dried at ambient temperature, and 200 g of leaves was subjected to hydrodistillation in a Clevenger-type apparatus for 6 h.¹⁰ Leaf essential oils were stored in airtight containers at 4 °C prior to further analysis.

Thermal Stability of Leaf Essential Oils. Essential oils (20 μ L) from five provenances of *C. osmophloeum* (COA–COE) were added to 20 mL vials with alumni caps and septa, respectively. Samples were heated at 100 °C for 0.5, 1, 2, and 4 h. In addition, essential oil from COB leaf was added to 20 mL vials and heated at 25, 40, 60, 80, and 100 °C for 8 h, respectively, to evaluate their thermal stability.

Effect of Antioxidants on the Thermal Stability of Cin. Variances of components on thermal oxidation of Cin were studied. Cin (20 μ L) was added to 20 mL vials with alumni caps and septa. Samples were heated at 100 °C for 0.5, 1, 2, 4, and 8 h. In addition, seven commonly used antioxidants, including carvacrol, thymol,

eugenol, α -tocopherol, propyl gallate (PG), octyl gallate (OG), and butylated hydroxytoluene (BHT), were selected as additives for Cin. The concentration of the antioxidants used was 0.04 M in Cin. Samples (20 μ L) were heated at 100 °C for 4 h. Furthermore, different amounts of eugenol were added to Cin and essential oil. The ratios of eugenol/Cin were 0.33, 0.66, 1.32, 2.60, and 4.76% (v/v), and the ratios of eugenol/essential oil COB were 0.62, 1.23, 2.44, and 4.76%. Each sample (20 μ L) was heated at 100 °C for 4 h.

GC–MS Analysis. Electron impact mass spectrometric data were collected by PoLarisQ Ion Trap GC/FID/MSn system (Thermo, Waltham, MA) interfaced with a Trace GC Ultra equipped with a fused silica column (DB-5 ms, 30 m \times 0.25 mm, 0.25 μ m, Agilent J&W Scientific, Santa Clara, CA). The injection temperature was 250 °C. The temperature program of the column was started at 60 °C, was held for 1 min, and was then ramped at 4 °C/min to 150 °C. It was then programmed to ramp at 10 °C/min to 250 °C and held for 1 min. Helium was the carrier gas with a split ratio of 10:1 and flow rate at 1 mL/min. The ion source temperature was 200 °C, and the sector mass analyzer was set to scan from 50 to 450 amu. Samples were examined in triplicate, and the Kovats retention indices were calculated for all constituents using a homologous series of *n*-alkanes C₉–C₂₁. The mass spectrum of each compound was identified by the National Institute of Standards and Technology (NIST) MS Search 2.0, Wiley/NBS Registry of Mass Spectral library 7.0, literature data,²⁶ and authentic reference compounds. The relative amount of each compound in each sample was quantified according to the peak area integrated by the analysis program (Xcalibur 1.4 SR1, Waltham, MA). Samples were diluted to 0.1 mg/mL with ethyl acetate, and 100 μ L of internal standard (IS, *n*-tridecane, 0.1 mg/mL in ethyl acetate) was added to the diluted sample (1 mL).

Gas Chromatography–Flame Ionization Detector (GC–FID)

Analysis. Gas chromatographies of samples were performed on an Agilent 7890A gas chromatograph (Santa Clara, CA) equipped with a fused DB-5 silica column (30 m × 0.25 mm, 0.25 μm). The flow rate of helium (carrier gas) was 1 mL/min, and a split ratio was 10:1. The flow rates of hydrogen, air, and makeup flow were 40, 400, and 25 mL/min, respectively. Temperatures of the injector and FID were 230 and 250 °C. The programmed temperature of the column varied from 80 to 140 °C (5 °C/min) and from 140 to 250 °C (30 °C/min) and then held for 5 min. The data were collected and analyzed using a ChemStation system (Agilent, Santa Clara, CA). Samples were diluted to 0.5 mg/mL with ethyl acetate, and 100 μL of internal standard (*n*-tridecane, 0.1 mg/mL in ethyl acetate) was added to the diluted sample (1 mL). Mixed sample solution (1 μL) was injected in GC–FID. The ratios of peak area of the compound/peak area of the internal standard were calculated and analyzed using linear regression. The thermal stabilities of the samples were evaluated by retention of Cin (RC), and the RC value was calculated according to the following equation:

$$\text{RC (\%)} = \frac{\text{peak ratio of Cin and internal standard (after treatment)}}{\text{peak ratio of Cin and internal standard (before treatment)}} \times 100$$

Statistical Analysis. All determinations were conducted in triplicate, and results were calculated as the mean ± standard error (SE) in this study. The Scheffé multiple comparison of the SAS statistical program was employed to analyze the difference in thermal stability of essential oil and Cin under the influence of various factors ($p < 0.05$).

RESULTS AND DISCUSSION

Composition of Essential Oils. Constituents and their relative content of leaf essential oils from five *C. osmophloeum* provenances are presented in Table 1. The major constituent of essential oil is Cin, and its content is 88.74, 95.25, 87.25, 81.46, and 68.44% in the five essential oils of COA–COE, respectively. Other minor constituents are benzenepropanal and benzaldehyde. The contents of benzenepropanal in the essential oils from COA to COE are 4.47, 0.96, 2.97, 3.88, and 0.71%, respectively. In addition, the contents of benzaldehyde in the essential oils from COA to COE are 3.47, 1.56, 3.63, 5.91, and 0.76%, respectively. However, only essential oil from COE has linalool, and its content is 14.38%. Trace eugenol was detected in the essential oil, and its content is 0.43, 0.00, 0.65, 0.28, and 1.26% in the essential oils from COA to COE, respectively.

Our results are consistent with the data reported by previous researchers. Fang et al.²⁷ claimed that *C. osmophloeum* leaf essential oils from primary forests of central Taiwan in 1978 contained high levels of Cin (85.27%). It also contained benzaldehyde (1.93%), linalool (0.70%), and eugenol (0.93%). Cheng et al.,¹³ collected *C. osmophloeum* leaf from Dapingting, Nantou, Taiwan, in September 2002 and reported that its essential oil contained abundant Cin (79.85%), with trace amounts of benzenepropanal (7.00%), benzaldehyde (5.35%), and eugenol (1.13%).

Thermal Stabilities of Leaf Essential Oils from Five Provenances of *C. osmophloeum*. Thermal stabilities of essential oils from five provenances of *C. osmophloeum* (COA–COE) were tested, and the results are presented in Figure 1. After heating at 100 °C for 4 h, the RC in the essential oils of COA, COC, COD, and COE is 94.8, 98.7, 89.1, and 98.6%, respectively. In contrast, the RC in the essential oil of COB is 82.7, 48.9, and 25.1% after being heated at 100 °C for 1, 2, and

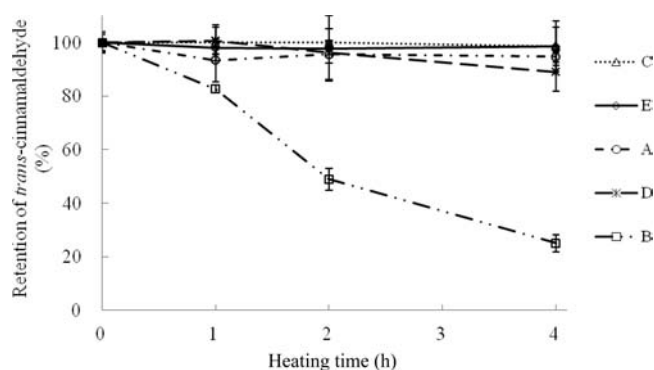


Figure 1. RC (%) in leaf essential oil from five provenances of *C. osmophloeum* after thermal oxidation. Results are the mean ± SE ($n = 3$).

4 h, respectively. These results revealed that essential oils from COA, COC, COD, and COE have great thermal stabilities but the thermal stability of the essential oil from COB is the worst.

The Cin contents in essential oils from five provenances of *C. osmophloeum* are all higher than 68%. In contrast, the contents of minor compounds in essential oil are different. We assumed that essential oils have great thermal stabilities because they contain eugenol, which has a phenolic group and a resonance structure. Eugenol can donate hydrogen and interrupt oxidation,²⁸ thus functioning as an antioxidant. The RC in the essential oil from COB, which does not have eugenol, decreased dramatically at 100 °C. On the other hand, the main constituents in the other oils became more stable, which might be attributable to the protection of eugenol against oxidation.

Effect of Temperatures on RC in Essential Oil.

Composition of essential oils from COB might be affected by temperatures, and RC in essential oil is shown in Figure 2. RC

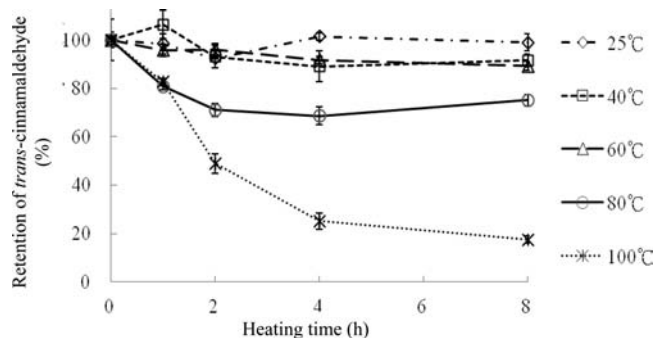


Figure 2. RC (%) of *C. osmophloeum* B leaf essential oil treated with different temperatures. Results are the mean ± SE ($n = 3$).

was 99% in COB when the essential oil was stored at 25 °C for 8 h. RC in COB was around 90% when the incubation temperature was raised to 40 and 60 °C for 8 h. In contrast, RC in COB decreased to 80% when the essential oil was heated at 80 °C for 1 h and stabilized after 2 h. RC in COB slightly decreased to 75.1% after being heated for 8 h at 80 °C. Significant decrement in the RC of essential oil was observed when it was heated at 100 °C. After heating for 1, 2, 4, and 8 h, the RC plunged to 82.7, 48.9, 25.1, and 17.4%, respectively. Results obtained demonstrated that *C. osmophloeum* leaf essential oil was stable when the heating temperature was lower than 60 °C but the content of Cin declined markedly when it was heated at 100 °C.

Qualitative and Quantitative Analysis of the Compound of Thermal Oxidation of Cin. The GC spectra of products from thermal oxidation of Cin were determined, and the retention time of Cin was 16.62 min. When Cin was heated at 100 °C, the peak area of Cin decreased significantly (data not shown). In addition, benzaldehyde, phenylacetaldehyde, acetophenone, cinnamic acid, and 1,2-dibenzoylthane were identified. The quantitative analysis results are shown in Figure 3. The contents of Cin are 868.5, 575.1, 495.5, 365.3, 283.1,

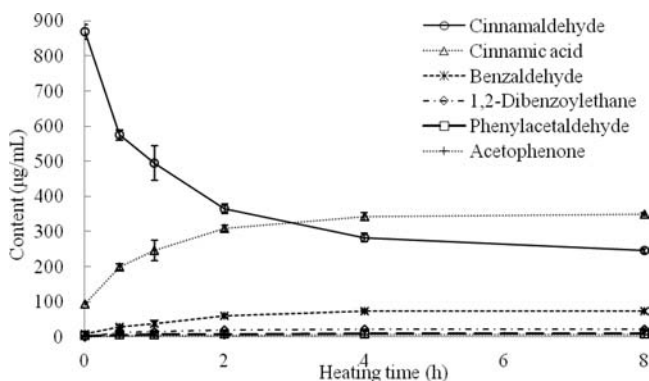


Figure 3. Main constituents in Cin after thermal oxidation. Results are the mean \pm SE ($n = 3$).

and 245.3 $\mu\text{g/mL}$ after heating for 0, 0.5, 1, 2, 4, and 8 h, respectively. However, the contents of the other degradation products increased when the heating time was prolonged. The contents of cinnamic acid and benzaldehyde were 94.2 and 8.8 $\mu\text{g/mL}$ before heating, respectively, but they increased to 349.2 and 72.9 $\mu\text{g/mL}$ after being heated at 100 °C for 8 h, respectively. In addition, the content of phenylacetaldehyde increased about 3 times compared to that at 0 and 8 h. The contents of 1,2-dibenzoylthane and acetophenone increased to 22.8 and 6.5 $\mu\text{g/mL}$ after being heated at 100 °C for 8 h.

Effect of Different Antioxidants on the Thermal Stability of Cin. In some commercial essential oils, antioxidants were employed to prolong their storage life. To improve the thermal stability of Cin, seven antioxidants were chosen, including carvacrol, thymol, eugenol, α -tocopherol, PG, OG, and BHT. The concentration of antioxidants employed in Cin is 0.04 M. All samples were heated at 100 °C for 4 h, and the RC in samples can be calculated after analysis by GC–MS. Results indicated that, without antioxidant, the RC decreased to 24% (Figure 4). All antioxidants could improve the thermal stability of Cin. Their protection effectiveness decreased in the order of eugenol, thymol, carvacrol, BHT, PG, α -tocopherol, and OG, with the RC of 62, 45, 40, 39, 35, 34, and 31%, respectively.

Similar results were reported in a previous study in which thermal stabilities of six essential oils were evaluated, including basil (*Ocimum basilicum* L.), cinnamon (*C. zeylanicum* N.), clove (*Syzygium aromaticum* L.), nutmeg (*Myristica fragrans* H.), oregano (*Origanum floribundum* M.), and thyme (*Thymus vulgaris* L.).²⁹ Contents of eugenol in basil, cinnamon, and clove essential oils were 14.2, 6.7, and 82.6%, respectively. It was observed that the main compositions in these essential oils did not change even after being heated at 180 °C for 3 h. These results proved that essential oils containing eugenol have better thermal stability.

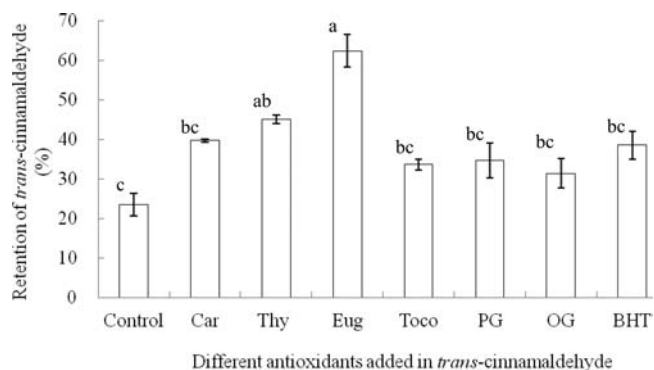


Figure 4. RC (%) by adding various antioxidants after heating at 100 °C for 4 h. Results are the mean \pm SE ($n = 3$). Cin, *trans*-cinnamaldehyde; Car, carvacrol; Thy, thymol; Eug, eugenol; Toco, α -tocopherol; PG, propyl gallate; OG, octyl gallate; and BHT, butylated hydroxytoluene. The concentration of antioxidants in Cin is 0.04 M.

Effect of the Eugenol Content on the Thermal Stability of Cin. Because the thermal stability of Cin can be improved by adding eugenol, it is imperative to determine the appropriate ratio of eugenol required in Cin. The ratios of eugenol in Cin examined in this study were 0.33, 0.66, 1.32, 2.60, and 4.76% (v/v). All samples were heated at 100 °C for 1, 2, 4, and 8 h, followed by analysis using GC–MS. The variations of RC are shown in Figure 5. Cin without eugenol

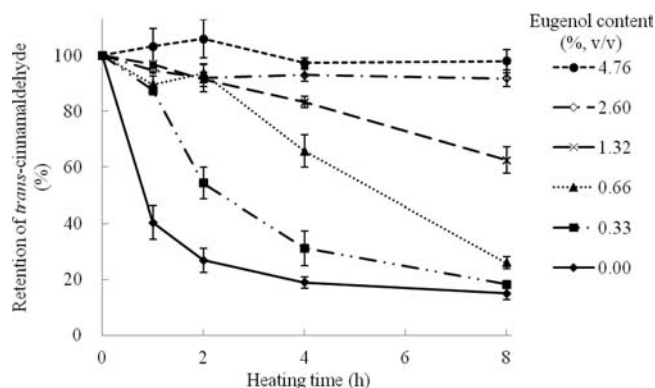


Figure 5. RC (%) by adding different amounts of eugenol in Cin after thermal oxidation.

decreased significantly when it was heated at 100 °C for 1, 2, 4, and 8 h, and its RC reduced to 40, 27, 19, and 15%, respectively. However, the RC in the sample with 0.33% eugenol was 88, 54, 31, and 18% when it was heated for 1, 2, 4, and 8 h at 100 °C, respectively. With 1.32% eugenol added, the RC was 83 and 63% when heated for 4 and 8 h. The thermal stability of Cin was significantly improved when 2.60 and 4.76% eugenol were added, respectively. Their RC was 92 and 98%, respectively, even after being heated for 8 h. It was concluded that, when a small amount of eugenol (0.33 and 0.66%) was added to Cin, its thermal stability could be improved after a short period (4 h) of heating, but the RC was below 26% after being heated for 8 h. When the content of eugenol added to Cin exceeded 2.60%, the thermal stability of Cin at a high temperature was significantly improved.

Similar results are also reported in a previous investigation. Friedman et al.³⁰ studied the effect of the temperature and time of heating duration on the decomposition of Cin. The relative content of Cin decreased to 63.1% after being heated at 100 °C

for 1 h. In contrast, Cin containing 5% eugenol displayed great thermal stability. The relative content of Cin was 88.1% after heating at 200 °C for 0.5 h.

Effect of the Eugenol Content on the Thermal Stability of Essential Oil. The thermal stability of Cin can be improved by adding eugenol. Therefore, the appropriate ratio of eugenol required in COB essential oil was investigated. The ratios of eugenol in essential oils examined in this study were 0.62, 1.23, 2.44, and 4.76%. All samples were heated at 100 °C for 1, 2, and 4 h, and the results are shown in Figure 6.

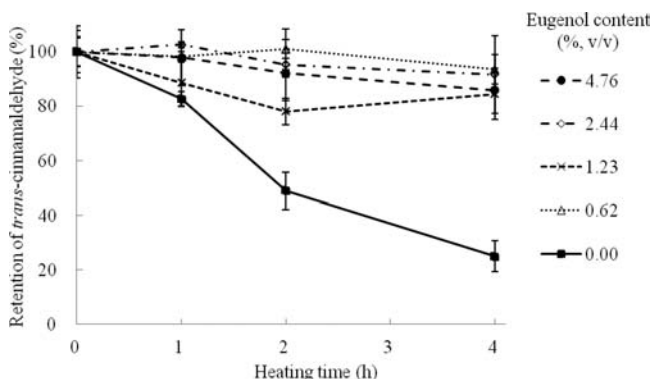


Figure 6. RC (%) in *C. osmophloeum* B leaf essential oil with different amounts of eugenol after thermal oxidation.

The RC of COB essential oil was decreased to 25% after being heated for 4 h. In contrast, the RC of essential oil with the addition of 0.62% eugenol was 94% after being heated for 4 h. In brief, the thermal stability of leaf essential oil can be improved by the addition of a small amount of eugenol.

When Cin and COB essential oil were heated at 100 °C for 8 h, their RC decreased significantly. However, the RC in COB essential oil decreased more slowly than the RC in Cin. When COB essential oil was heated for 1, 2, 4, and 8 h, the RC was 83, 49, 25, and 17%, respectively, while the RC in Cin decreased to 40, 27, 19, and 15%, respectively. The results showed that COB essential oil has better thermal stability than Cin, which might be due to minor antioxidant action provided by other constituents in the COB essential oil. Moreover, minor constituents in the oil might have synergistic effects with eugenol, so that thermal stability could be improved by a small amount of eugenol. There are trace α -pinene and camphene in COB essential oil. In previous research, with α -pinene and camphene, which are monoterpenoids proven to have slight antioxidant activity,^{31,32} the thermal stability of COB essential oil is better than the thermal stability of Cin.

In conclusion, *C. osmophloeum* and Cin can be widely used in food and cosmetic industries. However, their thermal stabilities have seldom been discussed. In this study, the thermal stabilities of leaf essential oil from five provenances of *C. osmophloeum* were examined, and their major component was Cin. The results indicated essential oil containing eugenol has better thermal stability than that without eugenol. The Cin content in essential oil without eugenol decreased significantly after heating at 80 and 100 °C. Seven antioxidants were added to Cin to improve its thermal stability, and eugenol showed the best activity. Thermal stability of Cin and essential oil can be improved when the ratios of eugenol were 2.60 and 0.62%, respectively. Application of our results to plant breeding helps improve the thermal stability of essential oil from *C.*

osmophloeum. Essential oil of *C. osmophloeum* could be made more stable by appropriate treatment or preservation, thereby increasing the economic value of this essential oil.

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Notes

The authors declare no competing financial interest.

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