

GC-MS analysis of volatiles in cinnamon essential oil extracted by different methods

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SUMMARY: Cinnamon essential oil (CEO) was extracted by three different methods: steam distillation (SD), ultrasound-assisted steam distillation (UASD) and microwave-assisted steam distillation (MASD). The volatiles in CEO were separated and identified by gas chromatography–mass spectrometry (GC-MS), and the differences in volatiles among the three different methods were further analyzed through principal component analysis. The results showed that 36 individual volatile components were present in the CEO from the three different methods. In general, the numbers of aldehydes, esters, alcohols, terpenes, aromatics and ketones were 6, 3, 7, 17, 2, and 1, respectively. The most abundant volatile component was determined to be cinnamic aldehyde. The content of total cinnamic aldehydes, which determines the price of CEO, was the highest among the three methods in the UASD sample (85.633%). Moreover, the highest yield (8.33‰) of essential oil was extracted by the UASD method. Therefore, UASD was the best way for CEO extraction in this research and was recommended for future industrial applications.

KEYWORDS: Cinnamic aldehyde; Cinnamon essential oil; Extraction method; GC-MS; Volatiles

RESUMEN: *Análisis de volátiles mediante GC-MS de aceites esenciales de canela extraídos por diferentes métodos.* El aceite esencial de canela (AEC) se extrajo mediante tres métodos diferentes: destilación al vapor (DV), destilación al vapor asistida por ultrasonido (DVAU) y destilación al vapor asistida por microondas (DVAM). Los volátiles del AEC se separaron e identificaron mediante cromatografía de gases-espectrometría de masas (GC-MS), las diferencias de los volátiles entre los tres métodos se analizaron adicionalmente a través del análisis de componentes principales. Los resultados mostraron la presencia de 36 componentes volátiles en el AEC mediante los tres métodos diferentes. En general, el número de aldehídos, ésteres, alcoholes, terpenos, aromáticos y cetonas presentes fue de 6, 3, 7, 17, 2 y 1, respectivamente. Se determinó que el componente volátil más abundante era el aldehído cinámico. El contenido de aldehído cinámico total, el cual decide el precio del AEC, en la muestra de DVAU (85,633%), fue el más alto entre tres métodos. Además, el mayor rendimiento (8,33‰) de aceite esencial se encontró mediante el método DVAU. Por lo tanto, DVAU fue la mejor forma de extracción de AEC en esta investigación y se recomienda en futuras aplicaciones industriales.

PALABRAS CLAVE: Aceite esencial de canela; Aldehído cinámico; GC-MS; Método de extracción; Volátiles.

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1. INTRODUCTION

Cinnamon (*Cinnamomum cassia Presl*), a tropical evergreen tropical tree from the *Lauraceae* family, is widely distributed in Southeast Asia (Chang, Chen, Chang, 2010). As the biggest producer of cinnamon in the world, China has produced more than 80% of the cinnamon in the world, especially in the Guangdong and Guangxi provinces, which accounted for 95% of the total production in China (Li *et al.*, 2013). Cinnamon is often used as a traditional medicine in India and China due to its unique medicinal and aromatic values, and it is mainly used for the treatment of anorexia, heart disease, intestinal disease and helminthic infections. Cinnamon bark and cinnamon essential oil (CEO) are included in pharmacopoeias in many countries (India, Britain, China, Australia, Belgium, France, Germany, Hungary, Japan, Portugal and Switzerland), and they have also been used as food additives, condiments and flavoring agents due to their carminative, antioxidant and preservative properties (Nabavi *et al.*, 2015). Rui *et al.*, (2009) found that the number of volatiles identified in *Cinnamomum cassia*, *Cinnamomum zeylanicum*, *Cinnamomum tamala*, *Cinnamomum burmannii*, *Cinnamomum pauciflorum* were 22, 22, 13, 6 and 21, respectively. Cinnamaldehyde, with good inhibitory effects on many food spoilage microorganisms, is the characteristic volatile component of CEO, which exists in all kinds of cinnamon species (Matan *et al.*, 2006). In addition, in international trade, the higher the content of total cinnamic aldehydes in CEO, the higher price of CEO is. Therefore, as the main volatile, the cinnamic aldehyde level should be obtained to the greatest extent during the extracting procedure of CEO.

Until now, the hydrodistillation, steam distillation (SD), steam and water distillation, maceration, hollow distillation, and expression methods have been widely used to obtain essential oils or extracts from plant materials (Jeyaratnam *et al.*, 2016). SD is a promising extraction method as its outstanding advantages such as solvent-free, easy to operate, and safe. In addition, SD can prevent volatile oils from decomposition because steam is able to reduce the boiling point of the oils (Wong *et al.*, 2014).

Furthermore, the ultrasound-assisted and microwave-assisted extraction techniques are also recognized as efficient methods with a short extraction time, increased yield and good quality (Cravotto *et al.*, 2008). Azlina *et al.*, (2013) extracted gaharu essential oil via ultrasonic assisted steam distillation (UASD), which increased extraction efficiency and reduced production costs. Microwave-assisted steam distillation (MASD) combines the advantages of both conventional and modern technologies, and is probably the leading technology in the

essential oil production industry. Golmakani and Rezaei (2010) increased extraction efficiency by 4 times using MASD compared to the traditional SD method during the extraction of *Zataria multiflora* essential oil.

At present, the SD method is used to produce CEO in factories. Since its disadvantages are low yield of essential oil, low total cinnamic aldehyde content in the products and high energy consumption, it is necessary to find a new extraction method with higher yield and cinnamic aldehyde content. In the present research, The UASD and MASD methods were used for comparison with the traditional SD method in the yield of essential oil and cinnamic aldehyde content as determined by Gas chromatography–mass spectrometry (GC–MS).

2. MATERIALS AND METHODS

2.1. Materials

Cinnamon (*Cinnamomum cassia Presl*) was collected from Yunfu City, (Guangdong Province, China) and naturally air-dried, then ground and screened into powder using a shaker with a 40-mesh sieve, and placed in a desiccator prior to use. Three samples were prepared for each treatment, for a total of 9 CEO samples.

2.2. Preparation of cinnamon essential oil by SD

Each cinnamon sample (150 g) was placed in a glass distillation flask (3.6 L). According to our former optimized process, where the power of a steam generator (self-made) was set at 600 W, and the vapor generated in the steam generator passed through the material for 2 hours, the CEO was separated from the mixture of water-oil by static stratification.

2.3. Preparation of cinnamon essential oil by UASD

The ultrasonic pre-treatment process was carried out in an ultrasonic cell crusher (JY92-IIN model, SCIENTZ, China). Each cinnamon sample (150 g) was put in a stoppered flask, and subjected to ultrasonic processing under settled conditions. Based on our former optimized process, ultrasonic power of 250 W and distillation temperature of 40 °C with a water to raw material ratio (w/w) of (16:1) for 25 min were applied. Then the samples were placed in glass distillation flasks (3.6 L) and the vapor generated in the steam generator passed through the material for 2 h, with the same conditions as SD.

2.4. Preparation of cinnamon essential oil by MASD

The microwave pre-treatment process was carried out in a microwave oven (P70D20N1P-G5 (W0) model, SCIENTZ, China). The essential oil

was obtained by MASD extraction according to our former optimized processing method. Briefly, each cinnamon sample (150 g) was put in a plugged glass flask, and subjected to microwave under settled conditions: a power of 400 W with a water to raw material ratio (w/w) (16:1) for 5 min. Then, the samples were put in the glass distillation flask (3.6 L) and the steam generated in the steam generator passed through the material for 2 h with the same parameters as SD.

2.5. The yield calculation

The yield of CEO was calculated by eq. 1.

$$\text{Yield (\%)} = \left[\frac{\text{the weight of CEO (g)}}{\text{weight of cinnamon sample (g)}} \right] \times 1000\% \quad (\text{Eq. 1})$$

2.6. GC–MS analysis

The volatile components were analyzed by GC-MS (7890A-5975C, Agilent, USA) using a HP-5 capillary column (The column was 30 meters in length with an inner diameter of 0.25 mm and 0.25 μm thickness) with 95% methyl and 5% dimethyl poly siloxane as the stationary phase. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The split ratio was 30:1 and the injected quantity was 0.2 μL . The program temperature conditions were: the oven temperature was maintained at 40 °C for 5 min, increased to 260 °C at a rate of 3 °C/min and held for 10 min, and then increased to 280 °C at 10 °C/min and maintained for 2 min. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage was adopted at 1823.5 V. The ion source was established at a temperature of 230 °C, the maximum temperature was set at 250 °C, and the quadrupole rod temperature was employed at 150 °C, with the maximum temperature of 200 °C. The mass range for this scanning was 50.0–550.0 amu. All volatile components were identified by matching the recorded mass spectra with the standard mass spectra provided by NIST11.L database.

2.7. Principal component analysis (PCA)

PCA was performed by SPSS 21.0 (SPSS Inc., Chicago, IL, USA) to identify the most important volatile components in the samples extracted by the three different methods explained above. $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. The yield of essential oil

According to Table 1, the UASD method showed the highest yield of CEO, followed by MASD. And the lowest yield was found for the SD method.

TABLE 1. The yield of Cinnamon essential oil (CEO)*.

Extraction Method	Yield of CEO (‰)
Steam distillation (SD)	3.91±0.09 ^a
Ultrasound-assisted steam distillation (UASD)	8.33±0.02 ^c
Microwave -assisted steam distillation (MASD)	5.53±0.03 ^b

*Different superscript letters mean significant difference ($p < 0.05$), $n=3$.

Significant differences in yield were seen among the three methods. The cell wall may have been damaged by the ultrasonic treatment so that the CEO was able to escape more easily; microwave treatment was beneficial for CEO extraction, but the effect was not as good as the ultrasonic treatment.

3.2. Volatiles separated and identified by GC–MS

The volatile components collected from the different methods were separated and identified by GC-MS. Total ionisation chromatograms (TIC) of the volatile constituents of CEO extracted via SD, UASD and MASD are shown in Figure 1(a–c) and the identification results of volatile components are listed in Table 2. Six kinds of compounds were identified, including aldehydes, esters, alcohols, terpenes, aromatics and ketones.

The TIC results (Figure 1) showed that the retention time of the volatile components ranged between 10 and 56 min, most of which were concentrated between 22 mins to 40 mins. Referring to the NIST11.L map library, 36 identical volatile components were confirmed in all the tested samples extracted by SD, UASD and MASD, indicating that extraction method had a minor influence on the varieties of volatiles in the CEO. Moreover, there were three major components in the tested samples, and the remaining components were minor.

Table 2 shows that there were six aldehydes in the volatile components. The relative contents of total aldehydes were 85.104%, 86.713% and 84.447% for the samples extracted by SD, UASD and MASD, respectively, indicating that aldehydes were the major components in the essential oil. Among the aldehydes, cinnamic aldehyde accounted for the majority of the contents (73.345%, 72.371% and 67.211% for SD, UASD and MASD, respectively). Cinnamic aldehyde plays a crucial role in imparting the characteristic flavor to cinnamon (Jayatilaka *et al.*, 1995), and it is a potent natural food preservative due to its antibacterial properties against five common foodborne pathogenic bacteria (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella anatum*) (Rui *et al.*, 2009; Ooi *et al.*, 2006). Apart from cinnamic aldehyde, 2-methoxycinnamaldehyde (10.611%,

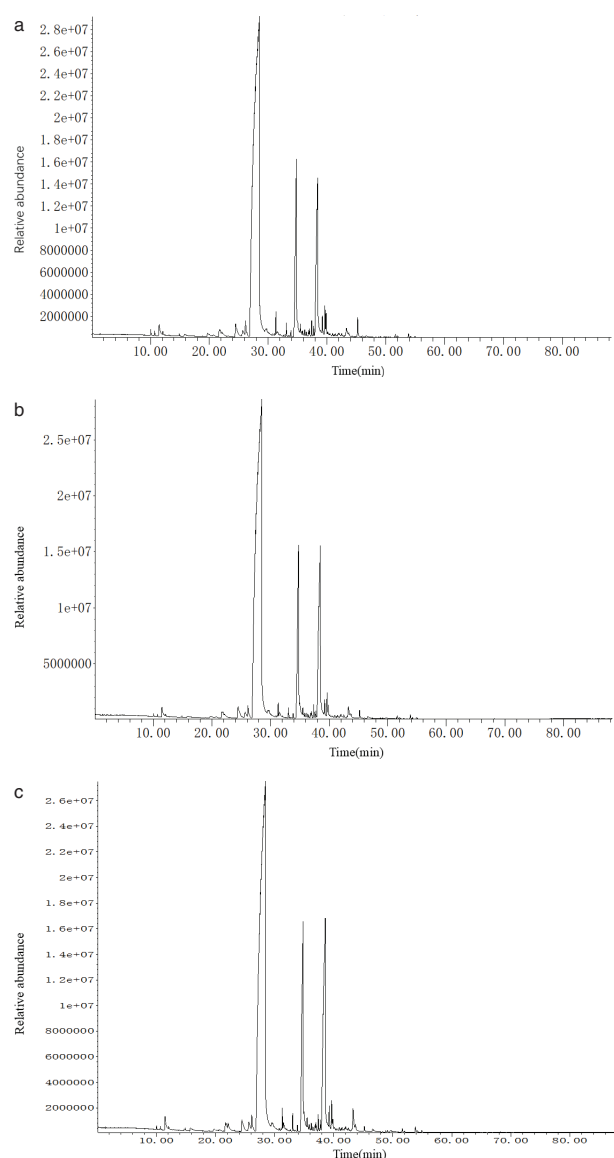


FIGURE 1. Total ion chromatograms (TIC) of cinnamon essential oil extracted by steam distillation (a), ultrasound-assisted steam distillation (b) and microwave-assisted steam distillation (c).

13.262% and 15.900%, respectively), benzaldehyde (0.513%, 0.455% and 0.536% for SD, UASD and MASD, respectively), and o-anisaldehyde (0.376%, 0.391% and 0.531% for SD, UASD and MASD, respectively) were the other aldehydes were present in high relative contents. Among the above aldehyde components, 2-methoxycinnamaldehyde is considered a crucial component of the unique antibacterial property in CEO (Chang *et al.*, 2001) and a potential agent for anticancer therapy (Wong *et al.*, 2016). The contents of total cinnamic aldehydes (including cinnamic aldehyde and 2-methoxycinnamaldehyde) were 83.956% (SD), 85.633% (UASD) and 83.110%

(MASD). Therefore, the highest total content of cinnamic aldehydes was obtained from UASD.

The analytical results were basically consistent with previous works, although there were differences in the number of volatile aldehydes between the present study and earlier studies (Rui *et al.*, 2009; Li *et al.*, 2010), which might be due to different cultivars. Furthermore, it was shown that the MASD extraction method produced the lowest cinnamic aldehyde, but yielded the highest 2-methoxycinnamaldehyde compared with the SD and UASD extraction methods. It might be due to the heating effect of the microwave, which led to the conversion of cinnamic aldehyde in the essential oil to 2-methoxycinnamaldehyde.

Yu *et al.*, found that many esters in cinnamon were valuable packaging materials (Yu *et al.*, 2007). Table 2 shows that three kinds of esters were identified in the CEO extracted by the three methods, i.e., phenylethyl acetate (0.478%, 0.413%, and 0.460% for SD, UASD and MASD, respectively), cinnamyl acetate (8.583%, 8.210%, and 9.553% for SD, UASD and MASD, respectively) and benzyl benzoate (0.094%, 0.124% and 0.152% for SD, UASD and MASD, respectively). Some researchers found that cinnamyl acetate and phenylethyl acetate were the main volatile substances that impart sweetness, fruitiness, floral aroma, and honey aroma to cinnamon essence ointments (Liet *et al.*, 2013). Moreover, the sample with the highest content of total esters (10.013%) and cinnamyl acetate (9.553%) was the CEO extracted by MASD. According to the report described by Jeyaratnam *et al.*, (2016), it might be attributed to the molecular polarization of the microwave, which leads to the extraction of oxygenates from CEO.

The results showed that there were 7 alcohols and 17 terpenes in the CEO, which is basically consistent with the work by Singh *et al.*, (2007). In summary, 7 alcohols identified from the CEO extracted by SD, UASD and MASD accounted for 2.300%, 2.025% and 2.325% of the total peak area, respectively; while 17 terpenes accounted for 3.206%, 2.260% and 2.724%, respectively. It was noteworthy that the content of each substance was less than 1%.

Other volatiles in the CEO were aromatics and ketones. The total amount of these compounds was less than 0.3% in the CEO, but they play vital roles in the aroma, antibacterial and antioxidant properties of CEO. For example, 6-methyl-5-hepten-2-one can endow CEO with the spicy flavor (Luna *et al.*, 2006), which is significant for the aroma of the commercial essence (Alonso *et al.*, 2009). As aromatics have phenolic groups, p-cymene and eugenol could be provided with definite antioxidant activities. In addition, researchers have also discovered that p-cymene is effective in suppressing conidial germination (Hong *et al.*, 2015) and the p-cymene-treated fruit can reduce levels of several anthocyanins

TABLE 2. Volatile components of cinnamon essential oil extracted by SD, UASD and MASD*

Code	Components	Molecular formula	Relative content (%) ^a		
			SD	UASD	MASD
1	α -Pinene	C ₁₀ H ₁₆	0.092±0.006	0.042±0.004	0.054±0.003
2	Camphene	C ₁₀ H ₁₆	0.078±0.007	0.037±0.003	0.048±0.002
3	Benzaldehyde	C ₇ H ₆ O	0.513±0.009	0.455±0.041	0.536±0.007
4	β -Pinene	C ₁₀ H ₁₆	0.107±0.005	0.044±0.004	0.090±0.001
5	6-Methyl-5-hepten-2-one	C ₈ H ₁₄ O	0.016±0.002	0.008±0.001	0.012±0.002
6	<i>p</i> -Cymene	C ₁₀ H ₁₄	0.017±0.003	0.013±0.002	0.029±0.002
7	D-limonene	C ₁₀ H ₁₆	0.053±0.006	0.034±0.006	0.047±0.002
8	Salicylaldehyde	C ₇ H ₆ O ₂	0.122±0.002	0.131±0.001	0.165±0.001
9	Phenethyl alcohol	C ₈ H ₁₀ O	0.202±0.005	0.168±0.003	0.151±0.004
10	Phenylpropyl aldehyde	C ₉ H ₁₀ O	0.205±0.005	0.180±0.001	0.217±0.005
11	Borneol	C ₁₀ H ₁₈ O	0.268±0.004	0.242±0.002	0.255±0.002
12	Cinnamic alcohol	C ₉ H ₁₀ O	0.319±0.002	0.311±0.002	0.435±0.002
13	Alpha terpineol	C ₁₀ H ₁₈ O	0.031±0.005	0.038±0.003	0.020±0.004
14	Cinnamic aldehyde	C ₉ H ₈ O	73.345±0.005	72.371±0.004	67.211±0.010
15	<i>o</i> -Anisaldehyde	C ₈ H ₈ O ₂	0.376±0.005	0.391±0.006	0.531±0.010
16	Phenyl ethyl acetate	C ₁₀ H ₁₂ O ₂	0.478±0.003	0.413±0.006	0.460±0.005
17	Trans -2-decenal	C ₁₀ H ₁₈ O	0.137±0.004	0.103±0.004	0.104±0.003
18	Cyclosativene	C ₁₅ H ₂₄	0.025±0.002	0.018±0.002	0.028±0.003
19	α -Ylangene	C ₁₅ H ₂₄	0.044±0.001	0.043±0.003	0.045±0.005
20	α -Copaene	C ₁₅ H ₂₄	0.341±0.001	0.209±0.002	0.266±0.002
21	Eugenol	C ₁₀ H ₁₂ O ₂	0.202±0.003	0.234±0.006	0.298±0.007
22	β -Elemene	C ₁₅ H ₂₄	0.027±0.001	0.022±0.003	0.025±0.002
23	Caryophyllene	C ₁₅ H ₂₄	0.227±0.007	0.170±0.001	0.250±0.007
24	α -Himachalene	C ₁₅ H ₂₄	0.109±0.001	0.087±0.002	0.091±0.007
25	Cinnamyl acetate	C ₁₁ H ₁₂ O ₂	8.583±0.353	8.210±0.009	9.553±0.006
26	2-Methoxycinnamaldehyde	C ₁₀ H ₁₀ O ₂	10.61±0.271	13.262±0.006	15.900±0.353
27	γ - Muurolene	C ₁₅ H ₂₄	0.451±0.002	0.365±0.002	0.407±0.001
28	α -Muurolene	C ₁₅ H ₂₄	0.232±0.003	0.186±0.004	0.244±0.005
29	α -Curcumene	C ₁₅ H ₂₄	0.194±0.002	0.160±0.002	0.205±0.007
30	β -Bisabolene	C ₁₅ H ₂₄	0.136±0.002	0.084±0.002	0.127±0.003
31	δ -Cadinene	C ₁₅ H ₂₄	0.324±0.001	0.258±0.003	0.283±0.007
32	α -Longipinene	C ₁₅ H ₂₄	0.190±0.001	0.146±0.004	0.198±0.004
33	Nerolidol	C ₁₅ H ₂₆ O	0.537±0.007	0.496±0.006	0.591±0.007
34	Spathulenol	C ₁₅ H ₂₄ O	0.738±0.035	0.590±0.030	0.656±0.036
35	Globulol	C ₁₅ H ₂₆ O	0.576±0.007	0.355±0.006	0.316±0.007
36	Benzyl benzoate	C ₁₄ H ₁₂ O ₂	0.094±0.006	0.124±0.003	0.152±0.004

*SD (steam distillation); UASD (ultrasound-assisted steam distillation); MASD (microwave-assisted steam distillation). ^aEach value was expressed as mean \pm standard deviation (n=3)

without any phytotoxic effect (Kordali *et al.*, 2008), which might contribute to its overall antioxidant capacity. Moreover, eugenol is the main substance in *C. zeylanicum* leaf oil, *C. pauciflorum* leaf oil and *C. burmannii* leaf oil, which were found to exhibit strong antibacterial effects (Ali *et al.*, 2005) and nematocidal activity (Park *et al.*, 2007).

3.3. Results of principal component analysis

The principal component analysis (PCA) has been widely applied in data mining to investigate the underlying structure and to extract the maximum information from large data matrices, so as to preserve as much complete data as possible (Lopez *et al.*, 2007).

In this work, PCA was carried out to interpret the differences in the volatile components of CEO extracted by SD, UASD and MASD. Table 3 lists 22 major components of the tested samples. Table 4 shows that the cumulative contribution percent of variance of the first two principal components (Eigen values >1) was 97.744%, where the first principal component accounted for 55.666% and the second principal component accounted for 42.078%. The first principal component was found to be significantly relevant to phenyl ethyl acetate (0.997), borneol (0.973) and γ -muurolene (0.966), while the second principal component was strongly characterized by salicylaldehyde (0.996), cinnamic aldehyde (0.995), *o*-anisaldehyde (0.993) and eugenol (0.991).

Figure 2 (loading plot) and Figure 3 (scores plot) show the score distribution of the first two principal components. Both figures show clear separations among the CEO extracted by SD, UASD and MASD. The PCA figure of SD was positioned at the lower right quadrant of the biplot, which was characterized by globulol, phenethylalcohol and δ -cadinene, compared to UASD and MASD. The MASD was positioned at the upper left area of the score plot and close to the positive half of second principal component, dominated by the presence of cinnamyl alcohol and *o*-anisaldehyde. The components obtained by UASD appeared on the score map in the lower left area with cinnamic aldehyde.

TABLE 3. Loadings of volatiles in the first two principal components

Compounds	Component matrix Component		Rotated component Matrix ^b	
	1	2	1	2
Benzaldehyde	0.916	0.065	0.760	0.516
Salicylaldehyde	0.615	-0.784	-0.002	0.996
Phenethyl alcohol	-0.130	0.983	0.506	-0.853
Phenylpropyl aldehyde	0.993	0.025	0.796	0.595
Borneol	0.647	0.751	0.973	-0.190
Cinnamic alcohol	0.789	-0.604	0.247	0.963
Cinnamic aldehyde	-0.651	0.754	-0.045	-0.995
<i>o</i> -Anisaldehyde	0.706	-0.707	0.117	0.993
Phenyl ethyl acetate	0.818	0.573	0.997	0.056
α -Copaene	0.574	0.808	0.951	-0.280
Eugenol	0.520	-0.852	-0.118	0.991
Caryophyllene	0.994	0.062	0.819	0.566
Cinnamyl acetate	0.894	-0.380	0.467	0.851
2-Methoxy cinnamaldehyde	0.358	-0.918	-0.287	0.942
γ -Muurolene	0.625	0.768	0.966	-0.216
α -Muurolene	0.987	0.147	0.866	0.496
α -Curcumene	0.998	0.108	0.843	0.527
δ -Cadinene	0.539	0.839	0.942	-0.326
α -Longipinene	0.973	0.197	0.886	0.447
Nerolidol	0.968	-0.251	0.605	0.796
Spathulenol	0.603	0.743	0.933	-0.211
Globulol	0.044	0.999	0.653	-0.757

^b Rotation converged in triplicate.

TABLE 4. Percentage of variance and cumulative variance explained by the principal components

Component	Initial eigen values			Extraction sums squared loadings			Rotation sums of squared loadings		
	Total	Percentage of variance	Cumulative percentage	Total	Percentage of variance	Cumulative percentage	Total	Percentage of variance	Cumulative percentage
1	12.247	55.666	55.666	12.247	55.666	55.666	11.102	50.464	50.454
2	9.257	42.078	97.744	9.257	42.078	97.744	10.402	47.281	97.744

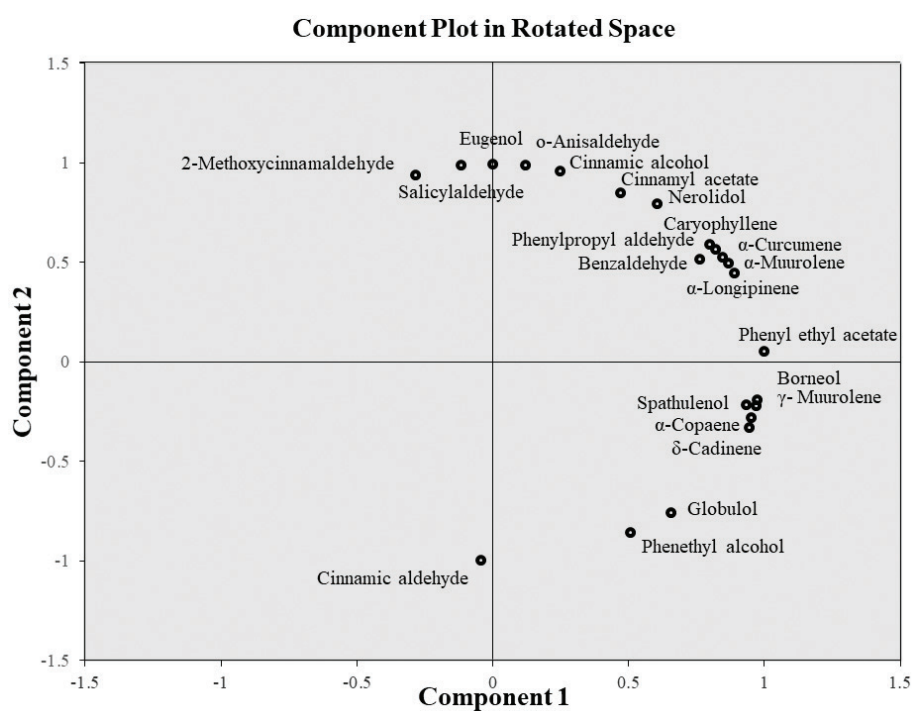


FIGURE 2. Loading plots after principal components analysis of the variables in the plane defined by the two first principal components.

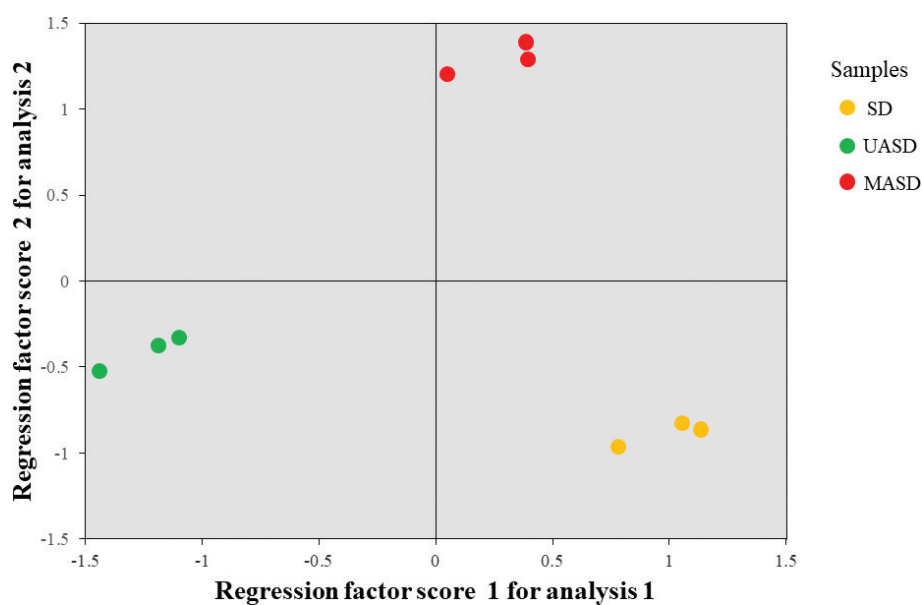


FIGURE 3. Scatter plot of scores via principal component analysis on the individuals in the plane defined by the two first principal components*

*SD (steam distillation); UASD (ultrasound-assisted steam distillation); MASD (microwave-assisted steam distillation)

4. CONCLUSIONS

36 kinds of volatile components were identified in CEO. UASD contained the highest content of total cinnamic aldehydes and the highest yield compared to the SD and MASD methods. Therefore, the UASD method is recommended for future industrial application.

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