

Quality attributes and chemical composition of commercial cinnamon oils

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RESEARCH ARTICLE

Abstract

Cinnamon oil is commercially available and has been used by the food, fragrance and pharmaceutical industries. In present work, 22 batches of commercial cinnamon oils from different brands were investigated. There are no significant differences in the appearance profiles and physicochemical properties between the samples, but great variation in the content of cinnamaldehyde was found by gas chromatography quantitative analysis. They were further chemically characterised by gas chromatography-mass spectrometry (GC-MS), and 54 volatile compounds were identified. According to principal component analysis of the GC-MS data set, the samples were clustered into three distinct groups with higher concentrations of cinnamaldehyde, diethyl phthalate, and eugenol as their markers, respectively. Moreover, coumarin, a hepatotoxic natural compound, was detected in some samples, which indicated that the samples with higher level of cinnamaldehyde and limited amount of coumarin would be better choice for efficacious and safe use.

Keywords: chemical components, commercial cinnamon oils, GC/GC-MS, PCA, quality attributes

1. Introduction

Cinnamon oil is commonly extracted from the barks, twigs and leaves of *Cinnamomum cassia* Presl or *Cinnamomum verum* Presl by hydro-distillation (Janick and Whipkey, 2007; Ravindran *et al.*, 2004). With the increasing use by the food, fragrance and pharmaceutical industries, its quality control has received many concerns from the public and health authorities. Generally, cinnamon oil is graded according to the percentage of cinnamaldehyde, which has been demonstrated varieties of bioactivities, such as anti-inflammatory (Lee *et al.*, 2002), anti-tumour (Ka *et al.*, 2003), anti-diabetic (Qin *et al.*, 2010), insecticidal (Liu *et al.*, 2014), anti-fungal (Guynot *et al.*, 2003), antimicrobial, and antioxidant activities (Singh *et al.*, 2007). In china, the content of cinnamaldehyde should be determined by gas chromatography (GC) method with no less than 75% of the oil (China Pharmacopeia Commission, 2015), while standards from the USA was 55 to 78% (United States Pharmacopieial Convention, 2014).

Previous research demonstrated that many factors, such as the species of *Cinnamomum*, the parts of the plant,

habitats, harvest seasons, storage circumstance, and the extraction methods, etc. (Figueiredo *et al.*, 2008; Geng *et al.*, 2011), have significant influence on the quality attributes of cinnamon oil. Among them, the botanical origin seems to be the most significant. For example, cinnamon oil from *C. cassia* contains more of the aromatic compounds compared to the one from *C. verum*. Coumarin and δ -cadinene are primarily present in *C. cassia*, but they are either absent or in low concentration in *C. verum* (Miller *et al.*, 1996). The major component in *C. verum* bark essential oil is cinnamaldehyde, while the main component in *C. verum* leaf essential oil is eugenol (Fichi *et al.*, 2007; Li *et al.*, 2016; Unlu *et al.*, 2010). Thus, cinnamon oils could be classified as different types depend on their chemical compositions.

Even though cinnamon oil has been extensively analysed, there is lack of information on quality assessment of the commercial cinnamon oils (CCOs). Quantitative differences among CCOs could result from not only the different species or parts but also brand factor. In the general markets, different brands of CCOs can be easily obtained, but their botanical origins are either uncertain

or unlabelled. Moreover, there is limited knowledge about their chemical components. It is difficult to grade them using the available approach only. In this work, 22 batches of CCOs (S1-S22) from different brands were investigated (Table 1). Their chemical components were characterised by gas chromatography-mass spectrometry (GC-MS), and then combined with principal component analysis to confirm associations between them. Organoleptic and physicochemical properties, and the contents of cinnamaldehyde were also determined. This study sought to provide important evidence for scientific grading of CCOs.

2. Materials and methods

Materials and sample solution preparation

Twenty-two batches of commercial cinnamon oils (S1-S22) were obtained from the general markets in China and listed in Table 1. The standards of cinnamaldehyde ($\geq 98\%$), 3-phenylacrylic acid ($\geq 98\%$) and coumarin ($\geq 98\%$) were purchased from Chinese National Institute for Food and Drug Control, Beijing, China. The samples (S1-S22) and the standards were dissolved in 2.0 ml of *n*-hexane respectively, and the solution was filtered through a 0.22 μm filter membrane.

Determination of physicochemical properties

Density was measured on a Qunlong high precision density meter DX-100G (Xiamen, China). Optical rotations were determined in MeOH solutions on a Anton Paar MCP200 digital polarimeter (Anton Paar, Ashland, VA, USA) at 25 °C.

Determination of cinnamaldehyde

GC analyses were performed using an Agilent GC-7890A gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with a HP-5 capillary column (30 m $\times$ 320 mm i.d., 0.25 μm film thickness; 5% phenyl methyl siloxane; Agilent J&W Scientific, Folsom, CA, USA). Carrier gas and flow rate: nitrogen with 1.0 ml/min. The oven temperature was programmed at 100 °C for 4 min, then increased to 150 °C at a rate of 5 °C/min and held for 5 min at 150 °C and finally to 200 °C at a rate of 5 °C/min and held for 5 min. Injector and flame ionisation detector (FID) temperatures were 200 and 220 °C, respectively. The column flow rate was 1.0 ml/min at a split ratio of 20:1.

The calibration graph is established by plotting the weight of standards against their peak area on the ordinate. The GC determination is proceeded by injection of 1.0 μl of

Table 1. Quality attributes of the samples (S1-S22).

Sample	Origin	Appearance		Physicochemical properties		Content (n=3, % $\pm$ SD) of cinnamaldehyde
	Brand (production date)	Aroma	Colour	Density	Optical rotation (°)	
S1	Oshadhi (2014.10)	strong aroma	orange	1.062	-0.81	82.75 $\pm$ 0.83
S2	Oshadhi (2015.01)	strong aroma	yellow	1.063	-1.20	89.83 $\pm$ 0.07
S3	Oshadhi (2015.06)	pleasant aroma	orange	1.043	-3.50	77.20 $\pm$ 0.90
S4	AromaShow (2014.09)	weak aroma	orange	1.065	-0.87	92.97 $\pm$ 0.63
S5	AromaShow (2015.04)	pleasant aroma	orange	1.056	-0.95	38.37 $\pm$ 1.75
S6	AA SKINCARE (2015.06)	pleasant aroma	pale yellow	1.030	-1.50	71.33 $\pm$ 0.44
S7	AromaShow (2015.10)	pleasant aroma	orange	1.066	-0.52	38.53 $\pm$ 0.97
S8	do TERRA (2015.02)	pleasant aroma	orange	1.067	-0.88	69.48 $\pm$ 1.01
S9	Oshadhi (2015.10)	pleasant sensation	yellow	1.039	-1.70	59.72 $\pm$ 1.14
S10	Florianna (2015.10)	pleasant aroma	pale yellow	1.056	-0.83	71.50 $\pm$ 1.12
S11	Oshadhi (2014.06)	weak aroma	yellow	1.037	-1.50	18.32 $\pm$ 0.03
S12	ZhenLian (2015.01)	weak aroma	orange	0.998	-1.60	95.78 $\pm$ 1.08
S13	Florial (2015.11)	strong aroma	yellow	1.048	-1.20	94.29 $\pm$ 1.80
S14	ZhenLian (2015.06)	pleasant aroma	pale yellow	1.055	-1.70	9.71 $\pm$ 0.09
S15	Tisserand (2015.01)	weak aroma	yellow	1.065	-0.75	10.77 $\pm$ 0.11
S16	Florianna (2015.03)	pleasant aroma	orange	1.039	-0.92	75.43 $\pm$ 0.30
S17	Wanhuayuan (2015.06)	strong aroma	orange	1.057	-1.60	91.13 $\pm$ 0.56
S18	Oshadhi (2014.03)	strong aroma	orange	1.054	-1.10	95.97 $\pm$ 0.52
S19	ZhenLian (2015.09)	pleasant aroma	pale yellow	1.057	-0.95	52.36 $\pm$ 0.23
S20	do TERRA (2015.08)	pleasant aroma	yellow	1.061	-0.92	77.12 $\pm$ 0.81
S21	Tisserand (2015.01)	strong aroma	yellow	1.067	-1.30	82.49 $\pm$ 0.74
S22	AromaShow (2015.07)	pleasant aroma	yellow	1.058	-1.70	59.24 $\pm$ 0.08

the sample solutions. The amount of cinnamaldehyde is obtained from calibration curve.

Linearity

Calibration curves consisted of six dilutions of cinnamaldehyde in *n*-hexane with concentrations of 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml. The peak area (*y*, mili absorbance unit) versus concentration (*x*, µg/µl) was analysed by linear least square regression, which resulted in $y = 1,088.3x - 874.79$ with a good correlation coefficient ($r=0.9998$).

Precision, repeatability and stability

In order to ensure the precision, a sample was subjected to GC analysis by 5 times with a relative standard deviation (RSD) value of 0.03% by calculating the peak area. Five parallel samples were prepared with the same method to examine the repeatability, and our results showed that all the peaks were presented at the same retention times, and RSD value of the peak area was 1.5%. Stability was presented by injecting in 0, 2, 4, 6, 8, 10 h with a RSD value of 2.3%. These results indicate that the method is reliable and applicable for the analysis of CCO.

Recovery

Recovery determination was as follows: the standard solution was added to the sample solution in triplicate, and the addition recovery of standard substance was determined. The average recoveries were estimated by the following equation:

$$\frac{\text{total detected amount} - \text{original amount}}{\text{added amount}} \times 100\%$$

The results show that the recovery of cinnamaldehyde is 99.71% (RSD 0.39%), which is adequate to meet the requirement of content determination.

Component identification

GC-FID and GC-MS analyses were performed under the same conditions as the above determination conditions, using a Agilent GC-7890A gas chromatograph equipped with FID system or the 5977A series mass selective detector in the electron impact mode (70eV; Agilent). MS source temperature: 230 °C; MS quadrupole temperature: 150 °C; mass scan range: 30-500 atomic mass unit.

The chemical components were identified by comparison of mass spectral patterns with those from the National Institute of Standards and Technology libraries data (NIST08, 11) provided by the software of the GC-MS

system, and by means of their retention index relative to the series of *n*-alkanes (C₈-C₄₀ alkane standard mixture; Merck, Darmstadt, Germany).

The relative concentrations of components were calculated from the GC peak areas obtain by GC-FID without using correction factors. The relative percentages are used only for comparative purposes to get different patterns of volatile profiles, and do not reflect the real amounts of compounds in the samples, since these values are obtained without any internal standardisation.

Principal component analysis

Using the SIMCA software (version 13.0; Umetrics AB, Umea, Sweden), principal component analysis (PCA) was carried out to find the association or difference between the samples. According to GC-MS data, the composition (%) of chemical components in each sample were used as the variables to feed the software, and the different samples were used as dependent variables for PCA treatment. Data were scaled using centring variable scaling format prior to PCA.

3. Results and discussion

Quality attributes of commercial cinnamon oils

The organoleptic (colour, aroma) and physicochemical (density, optical rotation) properties of the samples were determined. Results showed that they were pale yellow to dark orange, with pleasant, strong or mild aroma-spicy notes, similar densities ranging from 0.998 to 1.067, and similar optical rotations ranging from -0.52 to -3.50 which were closer to pure cinnamaldehyde (Table 1). There is no high variance in the appearance profiles and physicochemical properties between the samples, showing the difficulty of CCO's grading by the above properties.

Cinnamaldehyde contents in the samples were determined by GC (Figure 1). The results showed that the contents of cinnamaldehyde had high variance between the samples, within a wide ranging from 9.71 to 95.97% (Table 1). More than half of the samples could not meet the minimum levels set forth for cinnamon oil in the specifications of Chinese Pharmacopoeia. The observed variance of contents might result from the botanical origin related factors such as different species and parts, growing places, harvesting season and storage circumstance, which make it impossible to guarantee the quality consistence of CCO. Standardisation of the above related factors is thought to be a good approach for characterising the change of volatile constituents and has been used for the quality control of many herbal medicines preparations. To highlight its controlled quality and commercial competitiveness, CCOs should be accompanied with botanical origin and cinnamaldehyde content.

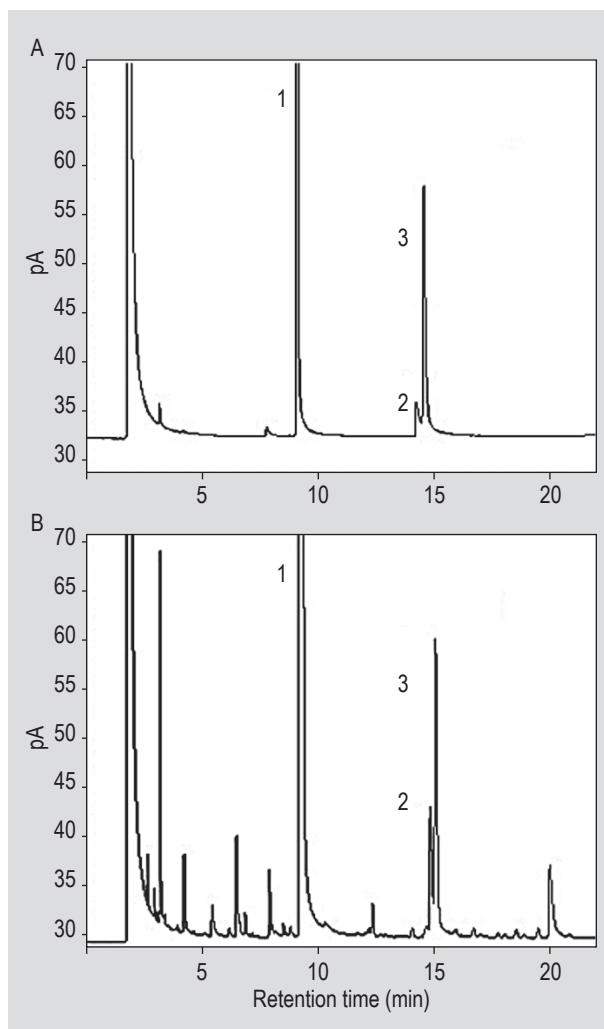


Figure 1. Typical gas chromatography chromatogram: (A) standards; (B) the sample S3; peak 1, 2 and 3 were assigned as cinnamaldehyde, 3-phenylacrylic acid and coumarin, respectively.

Chemical components of commercial cinnamon oils

Visual inspection of the total ion current chromatograms showed each samples' chromatogram was unique and characteristic, implying highly variant in chemical profiles among the samples (Figure 2). A total of 54 volatile compounds in CCOs (Supplementary Table S1), which accounted for 88.16–94.84% of the total composition, were tentatively identified. Samples S4 and S21 had the smallest number of components and sample S11 and S15 had the greatest. The main class of volatile compounds was aromatic compounds (56.29–93.36%) with the predominance of cinnamaldehyde (27) and eugenol (32), followed by monoterpenes (0.01–38.67%) with pinene (5) and copaene (34) as the major compounds, which suggested that CCOs on the general markets were phytochemically characterised with the abundance of aromatic compounds. These results are similar to those reported in the literature

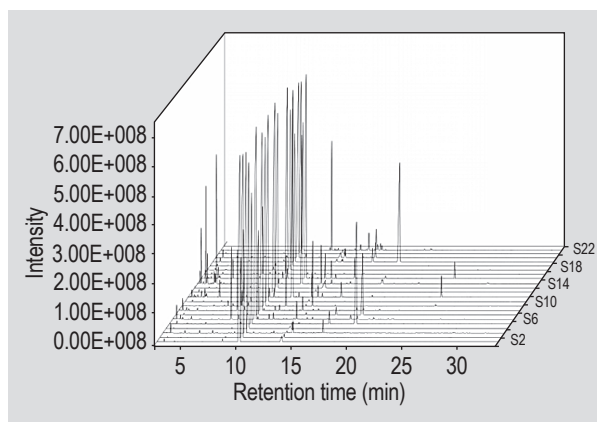


Figure 2. The total ion current chromatograms of the samples (S1–S22).

about this class of essential oils from cassia (Miller *et al.*, 1996). Only cinnamaldehyde (27) found in all the samples was assigned as their 'common components', while the minor components, such as 3-phenylacrylic acid (37), were quite variable and absent in several samples. These indicated the chemical components and their compositions of the samples were various from each other.

Scores plot of the PCA showed a good separation among the samples, and the separated three groups were named as CP1, CP2 and CP3 respectively (Figure 3A). Samples S5, S7, and S19 clustered into group CP1. Group CP2 included samples S11, S14, and S15. The rest of the samples clustered into group CP3. Furthermore, model validation revealed a predictive power of $Q^2(\text{cum})=0.593 (>0.5)$, which indicated the pattern recognition multivariate analysis was suitable for prediction.

The loading plot obtained for the same data set was corresponded to the PCA scores plot (Figure 3B). Diethyl phthalate (51), eugenol (32), and cinnamaldehyde (27) were farther away from the main cluster of components and should be the most influential components on the classification of the samples. The loading plot indicates that CP1 is characterised by higher concentrations of diethyl phthalate (51) as its marker component. In the same way, CP2 has the most content of eugenol (32). While, the contents of cinnamaldehyde (27) in the two groups were rather lower. The maximum content of cinnamaldehyde (27) was found in CP3, which determines the samples in this group a significant market competitiveness.

Although the botanical origins of the CCOs are unlabelled, GC-MS combined with PCA model works well to classify the samples according to their chemical composition. Previous studies have demonstrated that the components and contents of the volatile oils of *C. cassia* are significantly different among the barks, the leaves and the branches (Deng *et al.*, 2014; Li *et al.*, 2013), and even among barks

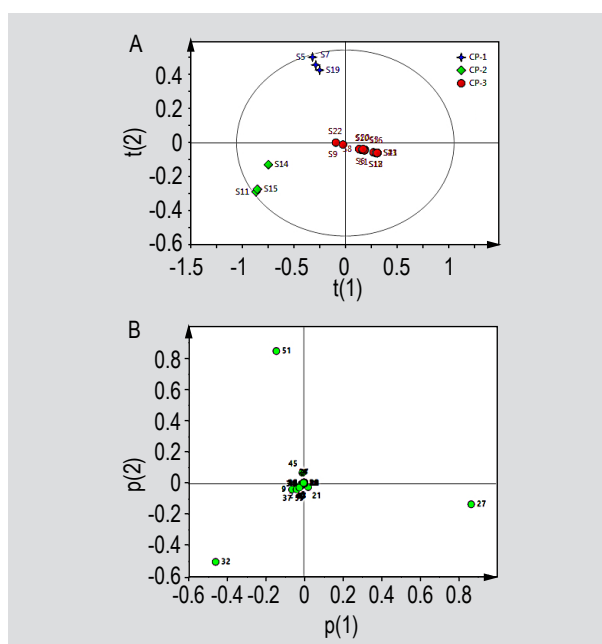


Figure 3. (A) Principal component analysis scores plot derived from the gas chromatography-mass spectrometry chromatogram data of the samples (S1-S22) with three varieties (assigned as group CP1, CP2 and CP3, respectively. Samples S5, S7, and S19 clustered into group CP1. Group CP2 included samples S11, S14, and S15. The rest of the samples clustered into group CP3). (B) Loadings plot obtained using centring scaling with mean centring.

at different ages (Geng *et al.*, 2011). It was also reported that cinnamaldehyde was the major component in *C. verum* bark essential oil, and eugenol was the main component in *C. verum* leaf essential oil (Fichi *et al.*, 2007; Li *et al.*, 2016; Unlu *et al.*, 2010). Thus, quantitative differences among the samples could be ascribed not only to brand factor but also to the different parts or collection factors.

Diethyl phthalate and coumarin in commercial cinnamon oils

Diethyl phthalate (DEP) appeared in large amounts in the samples of group CP1, and limited amounts in the samples of group CP2 (Table S1). However, DEP is not a natural compound, but a suitable solvent for many organic molecules, and often used to bind cosmetics and fragrances (Api, 2001). DEP is also widely used as a plasticiser in the manufacture of plastic packaging (Yang *et al.*, 2015). DEP has been reported to cause damage to the nervous system as well as to the reproductive organs (Ghorpade *et al.*, 2002; Miodovnik, 2011; Shanna, 2008). Moreover, some phthalic acid esters have been proved to be endocrine disrupting chemicals, and consequently United States Environmental Protection Agency and the European Union have placed six phthalic acid esters analogues in the priority pollutants list (Net *et al.*, 2015). To date, there are no records of detecting

DEP in cinnamon oils. Taking into account of the large amounts in the samples of group CP1, the addition of DEP in CCOs should be paid more attention.

Recently, there are many concerns about the content of coumarin in cinnamon containing products (Ballin and Sorensen, 2014). Coumarin was reported to have a connection with liver tumours in rats and mice (Felter *et al.*, 2006). It was also suspected to have genotoxic and carcinogenic effects in humans (Sproll *et al.*, 2008). A coumarin daily intake limit of 0-0.1 mg/kg body weight per day was recommended by the European Food Safety Authority in 2004. For special caramels and alcoholic beverages, the limit for coumarin was 10 mg/kg, and chewing gum with a limit of 50 mg/kg (EFSA, 2004). But so far, there are no reports on the composition of coumarin in CCOs. From Table S1, it could be found that coumarin was detected in sample S3, S5, S6, S7, S8, S11, S16, S19, S20, and S22, and showed a content ranging from 0.01 and 1.88%. If grading of the samples by higher level of cinnamaldehyde and limited amount of coumarin, all the samples in CP3 except S3, S6, S8, S16, S20, and S22 were better.

4. Conclusions

The quality variation of CCOs from the general market has been highlighted by the results of this investigation. The content of cinnamaldehyde in the samples varied significantly, ranging from 9.71 to 95.97%, so it is a challenge for CCOs to meet the product specifications. Different parts and collection factors have significant influence on the quality of CCOs, and could be discriminated by chemometric processing of GC-MS derived chemical profiles. According to the composition (%) of the identified 54 volatile compounds, the investigated samples are divided into 3 clusters, and cinnamaldehyde, diethyl phthalate, and eugenol were defined as their marker components. The PCA classification method for CCOs is reliable and feasible.

Additionally, it is not always important to have high amounts of components as long as CCOs have the desired or security profiles, since hepatotoxic natural compounds such as coumarin have been detected in the oils. It is impossible to remove all the coumarin in cinnamon oil because of high cost and time consuming. For efficacious and safe use, CCOs with higher level of cinnamaldehyde and limited amount of coumarin would be better choice. Application of cinnamaldehyde and coumarin as the quality markers, along with statistical data analysis methods, allows the identification of qualitative-quantitative differences among the samples, and provided important scientific evidence for grading CCOs on the general market.

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Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/QAS2018.1348>.

Table S1. The identified compounds and their compositions in the samples (S1-S22).

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