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# Characterization of odor-active compounds of various *Chrysanthemum* essential oils by gas chromatography–olfactometry, gas chromatography–mass spectrometry and their correlation with sensory attributes

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ABSTRACT

Volatiles of five kinds of *Chrysanthemum* essential oils with different manufactures were characterized by descriptive sensory analysis, gas chromatography–olfactometry (GC–O), gas chromatography-mass spectrometry (GC–MS) and statistics analysis. Six sensory attributes (floral, woody, grassy, fruity, sour and minty) were selected to assess *Chrysanthemum* essential oils. A total of 38 volatile compounds were detected and quantified using standard substances by GC–O and GC–MS. Terpenes constituted the largest chemical group among the volatiles of the essential oils. Then partial least squares regression (PLSR) was used to elucidate the relationship between sensory attributes and aroma compounds. The result showed that α-pinene, β-thujene, α-terpinolen, β-cubebene, caryophyllene, (*Z*)β-farnesene, (−)-spathulenol, linalool, camphor, camphene, 4-terpineol, *Z*-citral and 4-isopropyltoluene were typical aroma compounds covaried with characteristic aroma of *Chrysanthemum* essential oils.



## 1. Introduction

*Chrysanthemum* is found primarily in the majority regions of China [1,2]. More varieties of *Chrysanthemums* are cultivated, such as Shen-nong Sweet *Chrysanthemum*, Tender Huang-ju, Chamomile Flower, Hangzhou White *Chrysanthemum*, Florists *Chrysanthemum*, etc. [3–5]. The major quality attributes of *Chrysanthemum* are appearance, aroma and color [6]. However, the quality of *Chrysanthemum* and its market price are commonly determined by the aroma.

The volatile chemical compounds of *Chrysanthemum* essential oil are mainly composed of monoterpenes, sesquiterpenes, aldehydes, acids, esters and alcohols [7–9]. Recently, some researchers have paid more attention to the characterization of aroma compounds of flowers and essential oils. Xia et al. [10] used GC–MS to analyze the volatile chemical composition from *Chrysanthemum* indicum in Hubei Wufeng. The results showed that 81 kinds of ingredients were isolated from C. Indicum, L-camphor was highest content, which was up to 5.840%, followed by caryophyllene oxide 5.807%, (−)-bornyl acetate 5.551%, oleic acid 4.873% and endo-borneol 4.668%. Besides, HekmatSorush et al. [11] identified two different methods of Tanasetum parthenium (Iran), which were extracted with hydro-distillation and steam distillation by GC/MS. Li et al. [12] used GC–MS to quantify the essential oil constituents of *Tagetes erecta* L. fowers during the florescence from Heilongjiang. Rateb et al. [13] analyzed the comparative study of the essential oil content of the leaves and the flower heads using GC/MS, revealing the presence of 42 and 30 component with the major components camphor and chrysanthenyl acetate in the leaves and the flower heads.

Recently, some multivariate statistical methods, such as principal component analysis (PCA) [14,15], hierarchical cluster analysis (HCA) [16] and partial least squares regression (PLSR) [14,17,18], have been used to explore the relationships between sensory evaluation and chemical analysis of volatile compounds. However, to the best of our knowledge, the correlation between the volatiles of *Chrysanthemum* essential oil and its sensory attributes by PLSR is not available.

In this essay, we report our latest study of characterization of *Chrysanthemum* essential oils, which consisted of the following three steps: (a) investigating the sensory evaluation of *Chrysan-*

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*themum* essential oils; (b) identifying and quantifying volatiles of *Chrysanthemum* essential oils by GC–O and GC–MS; (c) using PLSR to study the relationship between sensory attributions and aroma compounds of five *Chrysanthemum* essential oil samples. It will help us to find the key volatile composition, which contributes to the characteristic aroma of *Chrysanthemum* essential oil.

## 2. Materials and methods

### 2.1. Materials

Five *Chrysanthemum* essential oils were purchased from different companies, including Shanghai Butterfly Trade Co., LTD (NO.1); Guangzhou Guowei Aromatic Technology Co., LTD. (NO.2); Jiangxi Ji'an Red Apple Natural Flavor Oil Co., LTD. (NO.3); Wuhan Yellow Crane Tower Essence Co., LTD. (NO.4); Nanchang Aroma Chemical Co., LTD. (NO.5).

### 2.2. Chemicals

Authentic standards of α-pinene(99.5%), camphene(99.5%), β-pinene(99.5%), sabinene(99.5%), β-myrcene(99.5%), α-phellandrene(99.5%), β-thujene(99.5%), dl-limonene(99.5%), *cis*-ocimene(99.5%), α-terpinolen(99.5%), β-cubebene(99.5%), caryophyllene(99.5%), β-farnesene(99.5%), β-guaiene(99.5%), δ-cadinene(99.5%), germacrene B(99.5%), cuparene(99.5%), neryl acetate(99.9%), dihydro methyl jasmonate(99.9%), linalool(99.9%), D-fenchyl alcohol(99.9%), 4-terpineol(99.9%), (−)-spathulenol(99.9%), eudesmol(99.9%), α-fenchone(99.9%), camphor(99.9%), (+)-carvone(99.9%), decanoic acid(99.9%), decanal(99.9%), *Z*-citral(99.9%), anisic aldehyde(99.9%), cinnamaldehyde(99.9%), 4-isopropyltoluene (99.9%), isoborneol(99.5%), borneol(99.9%), anethole(99.5%), thymol(99.9%) and carvacrol(99.5%) were purchased from Shanghai Titan technology Co., LTD. The *n*-alkane standard ($C_7$–$C_{30}$) was provided by Sigma–Aldrich Chemical Co. (St. Louis, MO). Analytical grade ethanol 99.7% (v/v) (Sinopharm Chemical reagent Co., Ltd.), anhydrous sodium sulfate was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Internal standard of methyl nonanoate was supplied by Sigma–Aldrich Co., Ltd. (St. Louis, MO, USA).

### 2.3. Sensory evaluation

Sensory evaluation was carried out in a sensory laboratory, which complied with International Standard ISO 8589:2007 [19]. The sensory analysis was performed by a well-trained panel consisting of 15 panelists (7 females and 8 males, age: 24–45). All panel members have passed screening tests according to the ISO standard. Screening tests chose 24 examples of odoriferous substances (such as D-limonene, butyric acid, vanillin and so on) that could be used for training, the assessor performed the assessment of the odor by sniffing the smelling strip, waving it gently a few centimeters from his/her nose. The strip should under no circumstances touch the nose, a mustache or the skin. Once a decision had been made, the assessor discarded the strip and replied to the questions on the answer form. The assessor then went on to examine the next substance. At last, the test supervisor interpreted the results, and eliminated those assessors who had made repeated errors. Prior to the quantitative descriptive sensory analysis, the panel members had thoroughly discussed aroma properties of samples through three preliminary sessions [20–22]. In the first step, panelists generated descriptive terms for the *Chrysanthemum* essential oils; in the second, different aroma standards were presented and discussed by panelists. From these discussions, the six aroma terms (floral, woody, grassy, fruity, sour and minty) were chosen for further descriptive analysis. In the third step, the *Chrysanthemum* essential oils were evaluated in triplicate using a 10-point interval scale (0 = none, 9 = extra strong). Then, the quantitative descriptive analysis was executed using 6 sensory attributes. The reference materials of aroma: floral (1 mg $L^{-1}$ aqueous solution of 2-phenyl ethanol), woody (5 g oak wood chips in 100 mL 10% ethanol–water solution), grassy (leaf, grass), fruity (crushed strawberries, raspberries and blackberries), sour (lemon, acid), minty (mint, coleus). Smelling strips were used to detect the odor. One end of a smelling strip (about 1 cm) was dipped into the sample. Three deep and quick sniffs were achieved from the smelling strip and then the odor source was removed. Clean air was breathed between each assessment. A gap of 20 s was sufficient between individual odor assessments [23].

### 2.4. Gas chromatography–olfactometry (GC–O)

GC–O was performed on an Agilent 7890 GC coupled to an Agilent 5973C mass selective detector and an olfactory detection port Gerstel ODP-2. One microliter of concentrated essential oil was injected on the 7890 GC. Samples were analyzed on a HP-INNOWAX column (60 m × 0.25 mm i.d. × 0.25 μm, J&W Scientific). At the end of the capillary column, the effluent was split into 1:1 for the MSD and sniffing port, using deactivated and uncoated fused silica capillaries as transfer lines, and the sniffing cone was purged with humidified air to help maintaining olfactory sensitivity by reducing dehydration of mucous membranes in the nasal cavity. The sniffing port was held at 250 °C to prevent any condensation of volatile compounds.

Detection frequency method (DFA) [24,25] using a panel of fifteen panelists (7 females and 8 males, 26 years old on average) was applied to obtain the odor profile of essential oils. The panelists were trained for one month in GC–O using 38 aroma-active standard compounds in a concentration 5 times above their odor thresholds in air. Whenever the aroma was detected by a panelist at the sniffing port in a GC run, then it was considered as "active" and the aroma descriptor was recorded. The sniffing time of each run was not more than 30 min. Determination of the odor descriptors detected by sniffing was carried out by triplicated experiments for each sample.

### 2.5. Gas chromatography–mass spectrometry (GC–MS)

Volatile compounds were analyzed by an Agilent 7890 gas chromatograph (GC) system coupled with a 5973C mass spectrometer (MS). HP-INNOWAX (60 m × 0.25 mm ID, 0.25 μm film thickness; J&W Scientific) analytical fused silica capillary column was used for chromatographic separations. 1 μL essential oil sample was injected into the injector port. Helium was used as the carrier gas at a velocity of 1 mL $min^{-1}$. Mass spectrum in the electron impact mode was generated at 70 eV and ion source temperature was 230 °C. The chromatograms were recorded by monitoring the total ion currents in 30–450 mass range. The oven temperature had been held at 50 °C, then ramped to 230 °C at the rate of 3 °C $min^{-1}$ and maintained for 10 min.

### 2.6. Identification and quantification of the volatiles

Identification of volatile compounds was based on matching their retention index (RI) and mass spectra with authentic standards. The retention index (RI) was used a homologous series of *n*-alkanes ($C_7$–$C_{30}$) to calculate. Quantification of volatiles was conducted on the HP-INNOWAX column by the external standard method. The standard curve of each volatile chemical was constructed using five different concentrations, which were contained