

Analysis of Volatile Compounds and Identification of Characteristic Aroma Components of *Toona sinensis* (A. Juss.) Roem. Using GC-MS and GC-O

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ABSTRACT

In this study, volatile compounds present in *Toona sinensis* (A. Juss.) Roem (TS) were investigated and their characteristic aromatic components were identified using Headspace Solid-phase Microextraction (HS-SPME) followed by Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Olfactometry (GC-O). The optimum conditions for extracting the volatiles from TS were achieved with the experimental parameters including the use of a 65 μm polydimethylsiloxane/divinyl benzene (PDMS/DVB) fibre, an extraction temperature of 40°C and an extraction time of 30 min. Under these conditions, 56 volatile compounds were separated and 53 were identified by GC-MS. Among them, 21 sulfide compounds (42.146%) and 27 terpenes (55.984%) were found to be the major components. The sample was analyzed by GC-O and 26 elutes were sniffed and their sensory descriptions evaluated by an odor panelists. Analysis of the data indicated, two compounds *cis* and *trans* isomers of 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene were major contributors to the characteristic aroma of TS.

Keywords: *Toona sinensis* (A. Juss.) Roem.; Volatile Compounds; Characteristic Aroma Components; HS-SPME; GC-MS; GC-O

1. Introduction

Toona sinensis (A. Juss.) Roem. (TS), is a tree commonly named Chinese toon or Chinese Mahogany and belongs to the family of Meliaceae. It widely distributes in China and some other Asian countries. Its tender leaves and young sprouts have been commonly used as a spice in China since the Han Dynasty around two thousand years ago. Due to its very unique flavour, TS is a popular traditional spice in the Chinese diet. The unique aroma can be sensed among almost all parts of TS, and provides a pleasant fragrance when its buds germinate. The edible buds are picked in the early spring annually, so TS is also recognized as a seasonal vegetable as well. As a fresh foliar vegetable, the delicious buds are usually cooked with other foods such as eggs and bean curds, in dishes known as *Scrambled eggs with Chinese toon* and *Tofu with Chinese toon*.

Almost every part of TS has been widely used in Chinese traditional medicine for treatment of enteritis, dysentery and itch during the period of ancient China [1].

Comprehensive investigations have demonstrated the pharmacological and health promoting properties of its high content flavonoid content [2,3]. Besides its medicinal functions, the most conspicuous trait of TS is its appealing flavours which are the results of its volatile compounds. However, limited studies are available on the volatile compounds in TS. Thus, experiments in this study have been designed to identify and characterize important volatile compounds in TS.

As a solvent-free extraction method, HS-SPME has shown many advantages for volatile compound analysis compared to traditional methods such as simultaneous distillation-extraction, and steam distillation [4]. For instance, the volatile compounds in garlic tend to be degraded during solvent extraction and steam distillation but not with HS-SPME [5]. In the analysis of volatiles from chili peppers, a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) extraction medium was showed to give the highest extraction efficiency with ground samples [6]. These studies also concluded that HS-SPME was a practical method to determine intermediate volatile compounds and even very

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volatile constituents produced enzymatically after the rupture of plant cells. Flavours present in TS is likely to be a mixture of very volatile and intermediate volatile compounds generated by physical cell disruption, thus, the HS-SPME technique may be a suitable technique for studying these compounds. GC-MS and GC-O have been widely used in flavour analysis and are very useful tools for identification of characteristic aroma compounds [7]. However, these combined techniques have not yet been applied to TS flavour analysis so far. Thus, this is the first attempt to use GC-MS and GC-O for TS flavour compound analysis after extraction using HS-SPME.

2. Materials and Methods

2.1. Chemicals

Chromatographically pure standards used for identification were supplied by several companies: (*Z*)-1-(Methylsulfany)-1-propene and (*E*)-1-(Methylsulfany)-1-propene were supplied by Heowns (Atlanta, USA). 2,5-Dimethylthiophene and 3,4-Dimethylthiophene were purchased from AccuStandard (New Haven CT, USA). An *n*-Alkane (C7-C33) mix in Hexane (Supelco, Bellefonte, USA) was also used to calculate the retention index (RI) of each component.

2.2. Sample Collection and Preparation

Fresh buds of TS were collected from Linfen city in Shanxi province, China, during spring, 2012. Before analysis, they were manually ground at room temperature using a mortar in order to release the fragrance adequately. A 10.0 g aliquot of sample was immediately transferred into a 40 ml gas-tight glass vessel and incubated at room temperature for 30 min to achieve partition equilibration for the volatile compounds.

Four extraction fibres; 30/50 μ m DVB/CAR/PDMS, 65 μ m PDMS/DVB, 75 μ m CAR/PDMS and 100 μ m PDMS (Supelco, Bellefonte, USA) were evaluated. Before extraction, the fibres were preconditioned for 30 min in the injection port of the GC as indicated by the manufacturer. The fibre was then inserted into the vessel and exposed to the headspace. The procedure was repeated using different extraction times (10 min to 50 min) and temperatures (10°C to 50°C) to determine the optimal HS-SPME conditions. Finally the fibre was removed and the components were desorbed in the GC injection port for 5 min.

2.3. GC-MS Operation Conditions

A Varian 4000 GC-MS (Walnut Creek, CA, USA) was used for separation and qualitative determination of the volatiles. Ultra-high purity helium was used as the carrier

gas at a flow rate of 1.5 ml/min into the column. Injection was at 250°C in split mode (20:1) onto a 30 m \times 0.25 m DB-5 capillary column with 0.25 μ m film thickness (Varian, USA). The oven temperature was set at 40°C for 3 min, then increased to 150°C at 4°C/min, and kept at this temperature for 1 min, ultimately increased to 260°C at 8°C/min, and kept at this temperature for 6 min. MS conditions were: ionization energy, 70 eV, full scan mode with a scan frequency of 1.2 scan/s and a scan range of 50 - 550 amu in all experiments and the transfer line was at 250°C. The ion trap was operated at 220°C in the electron impact mode.

2.4. GC-O Operation Conditions

The GC-O analysis was conducted using an Agilent 5975 gas chromatograph equipped with a FID detector (Agilent Technologies, Santa Clara, CA, USA) and a sniffing port (Sniffer 9000, Brechbühler, Switzerland). Injection and separation conditions were as indicated above. Gas chromatography effluents of TS extracts were split between the sniffing port and the FID at a ratio of 1:1. The temperature of the sniffing port was 240°C. The sniffing port was supplied with humidified air at 40°C with a flow of 600 ml/min in order to avoid nasal dehydration.

2.5. Sniffing Test

Sniffing tests were performed on eight chromatographic runs by four panelists (two males and two females in the age range of 20 to 30 years old). A panel consisting of 4 people was thought to be suitable for sniffing test based on previous studies [8,9]. An aroma evaluation approach outlined by Lv *et al.* [10] was used to describe aroma properties and intensities at the sniffing port under the conditions outlined above (see Section 2.4). According to the experience of previous TS flavour study, vocabulary pool for describing TS aroma was made. Prior to the experience, the panelists were familiarized with the pool of aromatic compounds and instructed on how to use suitable descriptors to describe the individual compounds in TS. They were asked describe the aroma properties and intensities of the compounds eluted from the GC column. An independent recorder was requested to write down the panelist's evaluation instantly. A compound was deemed as aromatically active if detected in at least half of all sniffs (four of eight runs). The intensity of each compound was evaluated by using a five-point intensity interval scale (1-very mild; 2-mild; 3-moderate; 4-strong; 5-very strong). A score was not given if no aroma was perceived. Finally the recorded data were collected, the mean values of related compounds were calculated to the nearest whole number (showed as the numbers of “*”) and the most frequent descriptions of each compound

were applied. The retention time and corresponding peaks were used to match the compounds measured in the GC-MS and GC-O system so as to determine the structure of the components sniffed in GC-O runs.

2.6. Qualitative and Quantitative Methods

Compound identification was confirmed on the basis of matching mass spectra of the database (NIST08, USA) and the odor descriptions of the related compounds (AD). Linear retention indexes (LRI) were also used to identify the compounds in the GC-O and GC-MS chromatograms. The retention index (RI) was determined using an n-Alkane mix at the same chromatography conditions and calculated according to the Kratz formula [11]:

$$RI = 100 \times n + 100 \times \frac{Rt(x) - Rt(n)}{Rt(n+1) - Rt(n)} \quad (1)$$

where $Rt(x)$ is the retention time of each targeted compound (x), $Rt(n)$ and $Rt(n + 1)$ are retention times of n-Alkane eluting directly before and after the compound (x) under the same chromatographic conditions. For difficulty to recognize optical isomers and *cis-trans* isomers, the retention time of the standard was applied for identification under the same chromatographic conditions.

The relative proportions of the constituents were obtained by peak area normalization. Quantitative results were obtained using the method as follows:

$$\begin{aligned} \text{Relative content (\%)} \\ = \text{single constituent area} / \text{total area} \times 100\% \end{aligned} \quad (2)$$

3. Results and Discussion

3.1. Optimal Extraction Conditions

Four fibres with different coating thicknesses and polar-

ity were used to extract volatiles from TS. The different extraction efficiencies are shown in **Figure 1**. The results showed that the 65 μm PDMS/DVB fibre was the best choice to extract volatiles from TS. Mu *et al.* (2007) also found this type of fibre absorbed the maximum number of TS flavour compounds compared to other types of fibre. The extraction temperature and duration were also investigated to improve the efficiency. The data are shown in **Figures 2(a)** and **(b)**. In general, extracting at 40°C showed the highest absorption of the flavour compounds. Extraction time studies indicated extraction efficiency was increased with absorption time from 10 to 50 min and almost reached the Max at 30 min. Consequently, a series of extractions at 40°C for 30 min were conducted using a 65 μm PDMS/DVB fibre to determine the characteristic aroma compounds of TS.

3.2. Precision of HS-SPME

The precision of the method was evaluated by performing 6 replicated runs of the TS sample using the optimum conditions. The relative standard deviation (RSD) values were calculated by the peak areas obtained by replicate analyses. As shown in the last column of **Table 1**, the calculated RSD values were less than 9%, which indicates the method consisting of HS-SPME followed by GC-MS and GC-O has an acceptable level of precision.

3.3. Volatile Compounds in TS

Volatile compounds identified in the TS sample by GC-MS and GC-O are summarized in **Table 1**. The table shows the chromatographic retention data (retention time and retention index) of each component in the GC-MS runs, the odor descriptions and intensities of the detected eluates given by the trained panel (including the identification method) and the similarity index of the

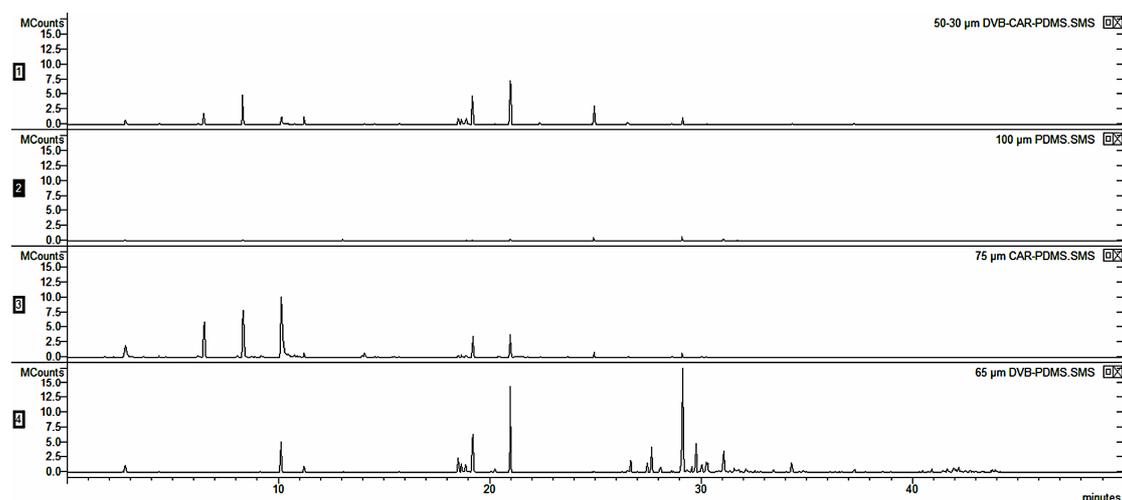


Figure 1. Influence of fibre type on extracting efficiency, the last type is 65 μm PDMS/DVB.

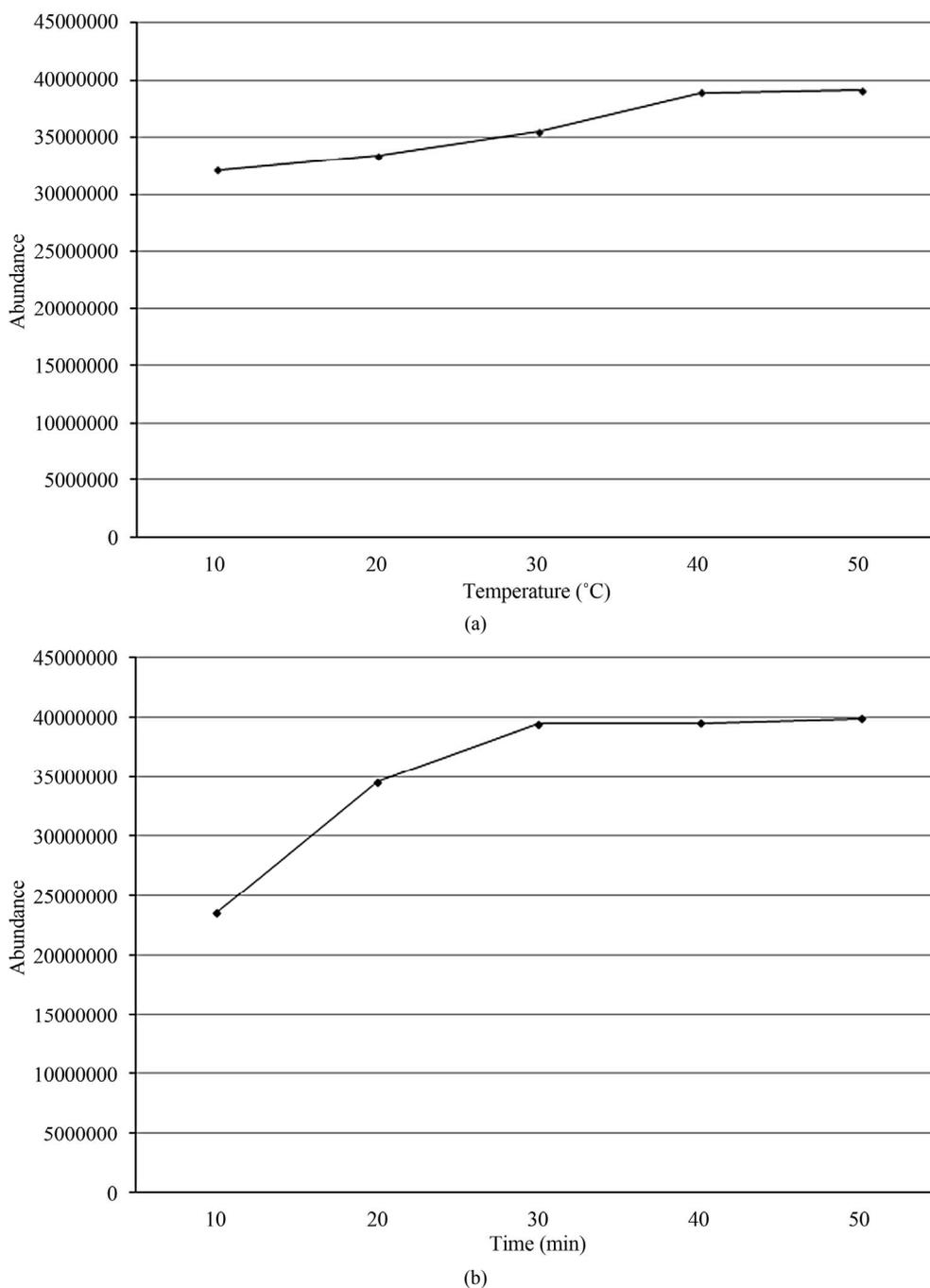


Figure 2. (a) Effect of absorption temperatures on peak area of TS volatile compounds using a 65 μm PDMS-DVB fibre for 30 min; (b) Effect of absorption time on peak area of TS volatile compounds using a 65 μm PDMS-DVB fibre at 40°C.

unknown compared with the spectrum of the MS database. Meanwhile, the relative amounts of these volatile compounds are also listed in **Table 1**. A total ion chromatogram of volatile constituents in TS is given in **Figure 3**.

The analysis of TS volatile compounds revealed a total of 56 compounds with 53 of these identified. The major classes included 24 terpenes (55.984%), 7 thiophenes

(32.660%) and 11 thioethers (8.764%). In addition, there were 2 aldehydes (0.049%), 2 thioesters (0.782%), 1 ester (0.296%), 1 thiapyrans (0.030%), and 1 ketone (0.010%). Among all the separated compounds, β -caryophyllene (21.450%) followed by *cis*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene (16.960%), *trans*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene (9.484%), 3,4-Dimethylthiophene (6.010%), Aristolene (5.413%), Germacrene D

Table 1. Volatile compounds present in TS determined by GC-MS and GC-O runs.

| NO. | Retention Time (min) | RI ^a | Compounds | Aroma property | Aroma intensity | MI ^b | Simil ^c | RC ^d | RSD ^e (%) |
|-----|----------------------|-----------------|---|---------------------------|-----------------|-----------------|--------------------|-----------------|----------------------|
| 1 | 2.762 | 610 | Methylthiirane | Fresh, garlic | *** | MS, RIL, AD | 780 | 1.902 | 6.47 |
| 2 | 4.357 | 696 | (Z)-1-(Methylsulfany)-1-propene | Garlic, onion | * | MS, RS, AD | 894 | 0.150 | 4.95 |
| 3 | 4.658 | 712 | (E)-1-(Methylsulfany)-1-propene | Garlic, onion | * | MS, RS, AD | 909 | 0.053 | 6.96 |
| 4 | 6.157 | 789 | 5-Methyl-2,3-dihydrothiophene | | | MS, RIL | 819 | 0.025 | 6.56 |
| 5 | 6.500 | 802 | Hexanal | Grass, leafy | ** | MS, RIL, AD | 790 | 0.002 | 2.31 |
| 6 | 8.341 | 863 | (E)-2-Hexenyl | Grass, leafy | ** | MS, RIL, AD | 872 | 0.047 | 5.21 |
| 7 | 9.146 | 876 | 2,5-Dimethylthiophene | Fried, onion, rubber | ** | MS, RS, AD | 900 | 0.130 | 2.34 |
| 8 | 10.133 | 887 | 3,4-Dimethylthiophene | Fried, onion, rubber | *** | MS, RS, AD | 905 | 6.010 | 2.17 |
| 9 | 10.456 | 894 | (Z,E)-Bis(1-propenyl)sulfide | Onion, garlic | ** | MS, RIL, AD | 761 | 0.031 | 5.25 |
| 10 | 10.746 | 901 | (E,E)-Bis(1-propenyl)sulfide | Onion, garlic | ** | MS, RIL, AD | 845 | 0.026 | 4.12 |
| 11 | 11.218 | 916 | α -Pinene | Fruity, resin | ** | MS, RIL, AD | 888 | 1.365 | 5.67 |
| 12 | 12.819 | 967 | α -Thujene | | | MS, RIL | 849 | 0.034 | 3.32 |
| 13 | 13.472 | 987 | β -Pinene | | | MS, RIL | 860 | 0.085 | 4.67 |
| 14 | 14.079 | 1005 | cis-2-Ethyl-3-methylthiophene | | | MS, RIL | 767 | 0.020 | 3.46 |
| 15 | 15.055 | 1030 | Limonene | | | MS, RIL | 874 | 0.083 | 3.56 |
| 16 | 15.726 | 1047 | cis-Ocimene | | | MS, RIL | 893 | 0.256 | 2.59 |
| 17 | 16.602 | 1070 | 3-Ethyltetrahydro-2H-thiopyran | | | MS, RIL | 789 | 0.030 | 4.69 |
| 18 | 17.981 | 1105 | 2-Ethenyl-1,3,3-trimethylcyclohexene | | | MS, RIL | 809 | 0.041 | 3.45 |
| 19 | 18.500 | 1119 | 3-Ethyl-1,2-dithiacyclohex-4-ene | Pungent, sulphur | ** | MS, RIL, AD | 845 | 3.721 | 7.56 |
| 20 | 18.677 | 1124 | 3-Ethyl-1,2-dithiacyclohex-5-ene | Pungent, sulphur | * | MS, RIL, AD | 789 | 0.941 | 6.54 |
| 21 | 18.853 | 1128 | 2-Ethyl-1,3-dithiacyclohex-4-ene | Pungent, sulphur | ** | MS, RIL, AD | 756 | 1.710 | 7.45 |
| 22 | 19.225 | 1138 | cis-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene | Cooked, TS, rubber | **** | MS, RIL | 845 | 9.484 | 4.65 |
| 23 | 20.051 | 1160 | Allyl dithiopropanoate | Onion, garlic | ** | MS, AD | 867 | 0.201 | 5.56 |
| 24 | 20.266 | 1166 | Prop-1-enyl dithiopropanonate | | | MS, RIL | 745 | 0.581 | 7.24 |
| 25 | 20.408 | 1170 | 2-Ethyl-5-propyl thiophene | | | | 754 | 0.031 | 6.14 |
| 26 | 20.990 | 1185 | trans-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene | Fresh, TS | ***** | MS, RIL | 827 | 16.960 | 4.44 |
| 27 | 21.313 | 1194 | 2-Ethyl-1,3-dithiacyclohex-4-ene | Pungent, sulphur | * | MS, RIL, AD | 748 | 0.101 | 4.78 |
| 28 | 22.793 | 1236 | 1,2-Dithiocane | | | MS, RIL | 856 | 0.007 | 5.21 |
| 29 | 23.704 | 1262 | 5,5-Dimethyl-1,3-dithian-2-one | | | MS, RIL | 611 | 0.032 | 8.45 |
| 30 | 24.958 | 1298 | Pelargonic acid methyl ester | | | MS, RIL | 787 | 0.296 | 3.43 |
| 31 | 26.267 | 1337 | δ -elemene | | | MS, RIL | 874 | 0.112 | 2.35 |
| 32 | 26.662 | 1349 | α -Cubebene | Herb | * | MS, RIL, AD | 877 | 3.426 | 2.79 |
| 33 | 27.456 | 1369 | Ylangene | Fruity, sweet | * | MS, RIL, AD | 849 | 1.815 | 5.63 |
| 34 | 27.668 | 1373 | α -Copaene | Cinnamon, floral | * | MS, RIL, AD | 886 | 4.414 | 7.45 |
| 35 | 28.093 | 1392 | α -Elemene | Flower, sweet | * | MS, RIL, AD | 867 | 1.486 | 5.43 |
| 36 | 28.605 | 1408 | (Z)-Caryophyllene | | | MS, RIL | 875 | 0.544 | 4.78 |
| 37 | 29.140 | 1425 | β -Caryophyllene | Fruity, sweet, flower | *** | MS, RIL, AD | 885 | 21.450 | 5.79 |
| 38 | 29.347 | 1431 | δ -Elemene | | | MS, RIL | 865 | 0.352 | 5.89 |
| 39 | 29.560 | 1438 | α -Guaiene: | | | MS, RIL | 881 | 0.727 | 7.46 |
| 40 | 29.770 | 1444 | Aristolene | Flower, sweet | * | MS, RIL, AD | 834 | 5.413 | 2.35 |
| 41 | 30.048 | 1453 | δ -Gurjunene | Flower, sweet | * | MS, RIL, AD | 864 | 1.688 | 4.45 |
| 42 | 30.251 | 1459 | α -Caryophyllene | Mild, mint | * | MS, RIL, AD | 900 | 3.162 | 2.67 |
| 43 | 30.849 | 1478 | γ -muurolene | | | MS, RIL | 874 | 0.122 | 5.21 |
| 44 | 31.095 | 1486 | Germacrene D | Oily, green | * | MS, RIL, AD | 911 | 4.856 | 8.08 |
| 45 | 31.588 | 1501 | α -Selinene | | | MS, RIL | 874 | 0.727 | 4.52 |

Continued

| | | | | | | | |
|----|--------|------|--|-------------|-----|-------|------|
| 46 | 31.799 | 1509 | α -Muurolene | MS, RIL | 883 | 0.345 | 7.01 |
| 47 | 32.119 | 1521 | γ -cadinene | MS, RIL, AD | 880 | 0.591 | 4.09 |
| 48 | 32.224 | 1525 | δ -cadinene | | 745 | 0.047 | 5.20 |
| 49 | 32.581 | 1538 | Selina-3,7(11)-diene | MS, RIL | 812 | 0.134 | 5.18 |
| 50 | 32.938 | 1552 | α -Calacorene | MS, RIL | 767 | 0.102 | 3.46 |
| 51 | 33.447 | 1570 | Germacrene B | MS, RIL | 854 | 0.607 | 4.67 |
| 52 | 34.025 | 1591 | Caryophyllene oxide | MS, RIL | 796 | 0.163 | 2.68 |
| 53 | 34.275 | 1601 | Unknown | | | 2.101 | 6.31 |
| 54 | 34.842 | 1628 | Unknown | | | 0.342 | 4.57 |
| 55 | 37.253 | 1754 | Unknown | | | 0.911 | 7.09 |
| 56 | 38.223 | 1812 | 2-Ethyl-3-hydroxy-1,4-naphthalenedione | MS | 799 | 0.010 | 3.67 |

^aRetention indices; ^bMethods of identification: MS, identified by MS; RS, identified by retention time of standard; AD, identified by aroma descriptions; RIL, identified by retention index and compared with those reported in the literatures; ^cSimilarity index of the unknown compared with the spectrum of the MS database; ^dRelative content by area normalization method; ^eRelative standard deviation of each component's RC.

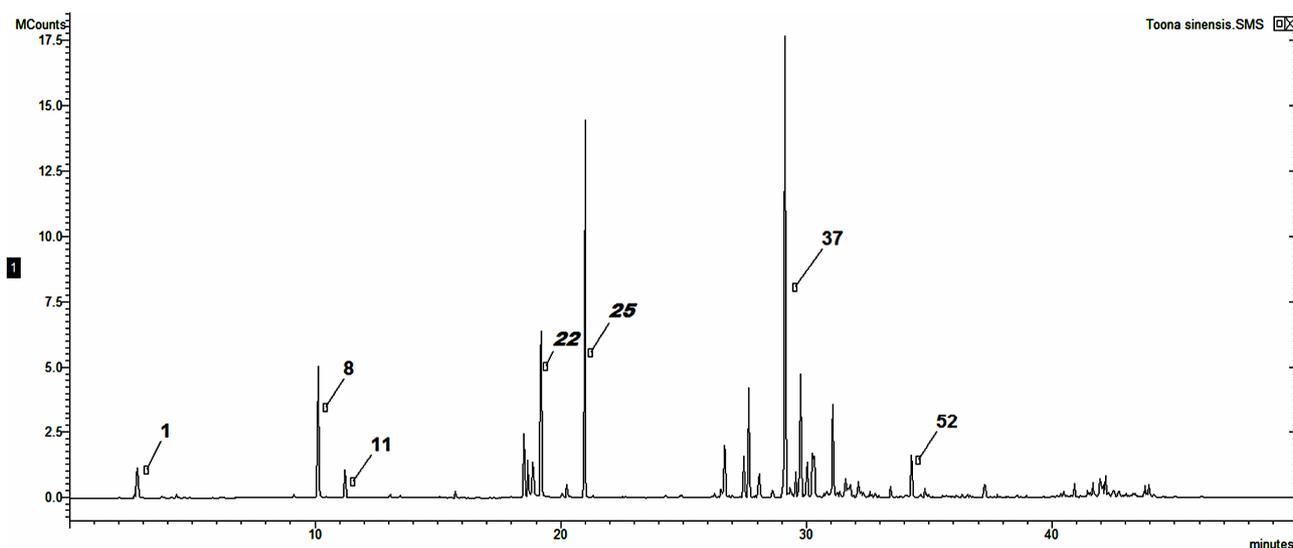


Figure 3. Total ion chromatogram of volatile components identified in TS. Peak numbers correspond to those listed in Table 1. Peak 22 and 25 were considered as the characteristic aroma of TS.

(4.856%), α -Copaene (4.414%), 3-Ethyl-1,2-dithiacyclohex-4-ene (3.721%), α -Cubebene (3.426%), α -Caryophyllene (3.162%), were found to be present in the highest concentrations.

The olfactometric strategy consisting of the measurement of aroma intensities and odor descriptions has been widely used [12,13]. In this study, the method was optimised to make method more convenient and accurate. For example, the use of an independent recorder was introduced to avoid the interruption between recording and sniffing. As shown in Table 1, GC-O analysis provided information about the impact of the major and subsidiary volatile components on the aroma of TS. A total of 26 components were considered aroma-active and their odor descriptions including aroma properties and intensities were evaluated by the panelists.

Terpene and sulfur compounds (especially terpenes with molecular weights of 204) thiophenes, thioethers were of the greatest importance to the aroma of TS (Table 1). Because of the high similarity in the MS of the terpene compounds, their structures were defined by calculating the retention index and comparing with the values in the database. Most of the terpenes found in TS can also be found in other foods such as carrot [14], grape and wine [15]. Anecdotal reports have suggested that terpene compounds were the major contributor to TS aroma. However, this study indicates these compounds are less important.

A total of 21 sulfur compounds were identified in TS extracts with thiophenes and thioethers representing the two most abundant groups of volatile compounds representing 32.660% and 8.764% of the total aroma (Table 1).

These sulfur compounds possess higher odor intensities and lower threshold values and have subsequently been confirmed to be the most important compounds of TS aromas. Large amounts of sulfur compounds can also be found in other foods such as cooked chicken [16], roast beef [17], heated leek [18], raw and processed garlic [19] and sliced onion [20].

Sniffing test of TS revealed 2,5-Dimethylthiophene and 3,4-Dimethylthiophene have an aroma of sulphur, fried onion and rubber (**Table 1**). Mu [21] also identified the two compounds in TS, but they did not describe their individual aromas. A small amount of Methylthiirane was found to be responsible for a “***” moderate pungent perception, and is also found to be one of the volatile components in garlic [22]. The thioethers, such as (Z)-1-(Methylsulfany)-1-propene, (E)-1-(Methylsulfany)-1-propene, (Z,E)-Bis(1-propenyl) sulfide and (E,E)-Bis(1-propenyl)sulfide are also found in onions and scallions [23]. Small amounts of aldehyde (0.049%) and ester (0.296%) compounds were also detected in TS (**Table 1**). It is not surprising to see that TS contains Hexanal and (E)-2-Hexenal, which is common in many plant extracts and are perceived as green and grassy odors [24]. Abundant terpenes give an aroma of sweet, fruit and flower, but the threshold of these compounds appear to be slightly higher than that of other compounds, such as sulfur compounds. Consequently, only those constituents with higher concentrations can be perceived. For example, β -Caryophyllene was found to have the highest concentration in TS, which is also present in many other plants such as clove [25] and cassia [26] as a major volatile compound. To the best of our knowledge, this study is the first report to identify a number of volatile compounds in TS, including *cis*-, *trans*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene, Allyl dithiopropanoate, α -Calacorene, Selina-3,7 (11)-diene, 3-Ethyl-1,2-dithiacyclohex-4-ene, 3-Ethyl-1,2-dithiacyclohex-5-ene, *cis*-2-Ethyl-3-methylthiophene, (Z)-Caryophyllene, α -Thujene 2-Ethyl-1,3-dithiacyclohex-4-ene, 2-Ethenyl-1,3,3-trimethylcyclohexene and 5,5-Dimethyl-1,3-dithian-2-one (**Table 1**).

3.4. Identification of the Key Aroma Compounds

The most important aroma compounds of TS, isomers of 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene (the identification method is discussed in the following paragraph), as indicated by peak 22 and 25, were first identified in this study. GC-O analysis indicated that the two peaks were demonstrated to be the characteristic peaks of TS. The two compounds eluted from the GC column one after another within two minutes, but their mass spectrograms seemed to be nearly identical (**Figures 4(a) and (b)**), which implied the hypothesis that they might be isomers.

Furthermore, the two mass spectrograms were difficult to identify even though many parallel tests were conducted (data not shown). By meticulously comparing the two mass spectrograms of 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene and 3,4-Dimethylthiophene with compounds in the NIST08 database (**Figures 4(a) and (b)**), the similarity of these compounds was noticed. This observation resulted in the hypothesis that peaks 22 and 25 corresponded to *cis* and *trans* isomers of 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene (**Figures 5(a) and (b)**). The presences of these two compounds may be due to the loss of H₂S from 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene to form 3,4-Dimethylthiophene which would explain the similarity of the MS for the two compounds. This hypothesis is supported by the study that demonstrated 3,4-Dimethylthiophene can be produced by from bis(1-Propenyl) disulfide in a series of reactions [27] (**Figure 6**). Nevertheless, because of the lack of studies in this area, the identity of the target compounds were unknown before the present study. According to Kuo & Ho [23] the two peaks were located on their corresponding positions, that is, the former is *cis*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene while the latter is *trans*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene. Referring to the flavour formation mechanism, 3,4-Dimethylthiophene and 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene scarcely exist in slicing onions and garlics, but the latter compound does not emanate its TS aroma in the allium vegetables, which may contribute to the other potent compounds such as allicin and allitride [28] and mask their odors. Due to the lack of the other pungent compounds and high amounts of 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene (both isomers, the former smelt like cooked TS while the latter showed its fresh TS aroma) it is likely that 3,4-Dimethylthiophene was available by heating 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene in garlic [29], which implied they might be generated from the same precursor in TS. However, the aroma chemistry in TS is very complicated, and requires further investigation. Although there have not been any reports on the mechanisms of TS aroma compound formation, a number of studies on compound formation in garlic, onions and leeks [30,31] suggest it may be possible to elucidate these mechanisms. For instance, some sulfur compounds act as aroma precursors, in that, as they are broken down via physiological metabolism, and aroma-active products are rapidly formed. Further investigation into the reaction mechanisms, related enzymes and substrates in TS is required.

4. Conclusions

This study indicated that HS-SPME coupled with GS-MS

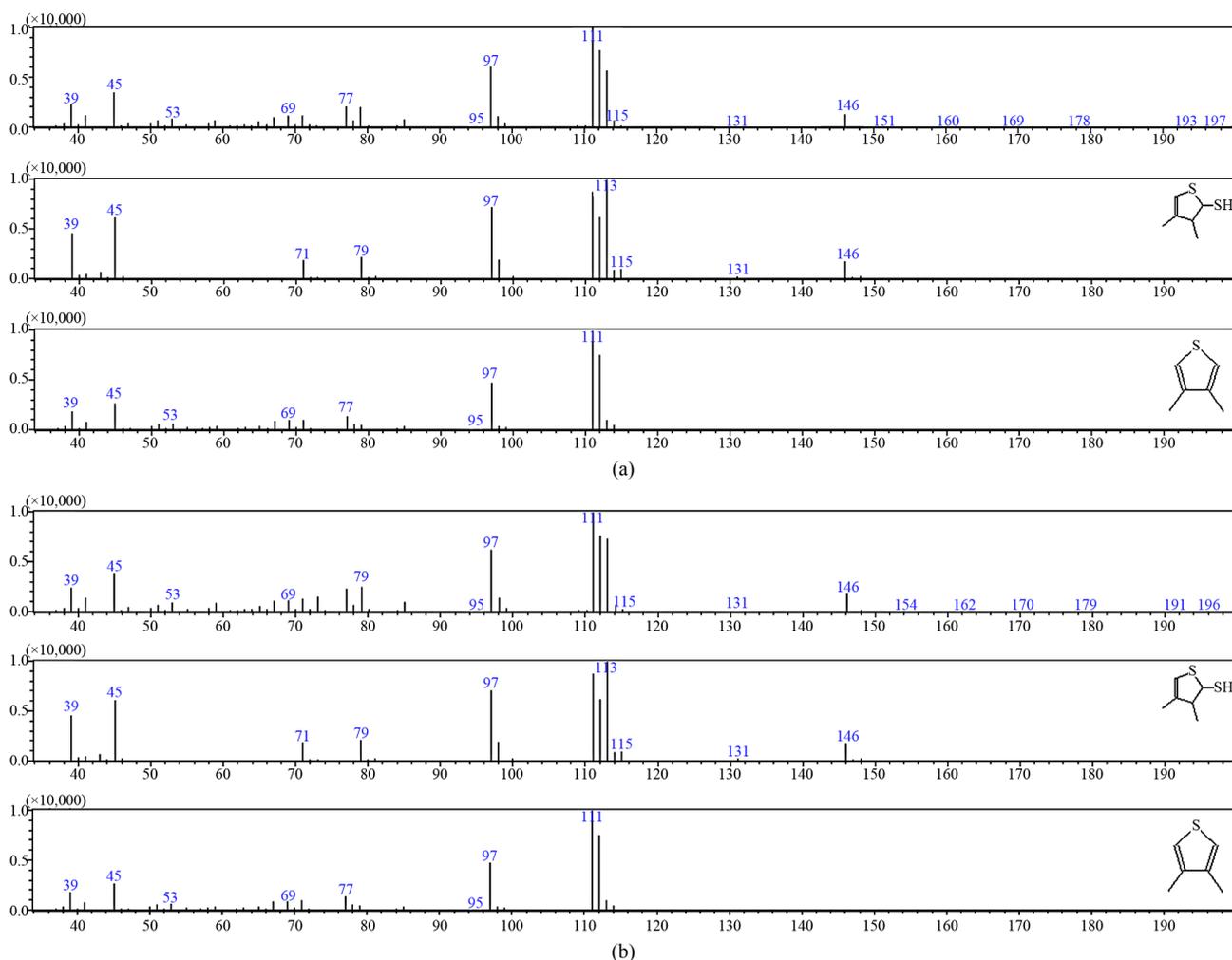


Figure 4. (a) Mass spectrogram of peak 22 comparing with 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene and 3,4-Dimethylthiophene; (b) Mass spectrogram of peak 25 comparing with 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene and 3,4-Dimethylthiophene.

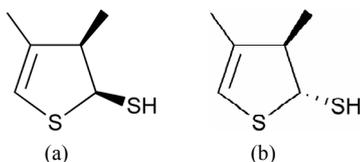


Figure 5. (a) The chemical structure of *cis*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene; (b) The chemical structure of *trans*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene.

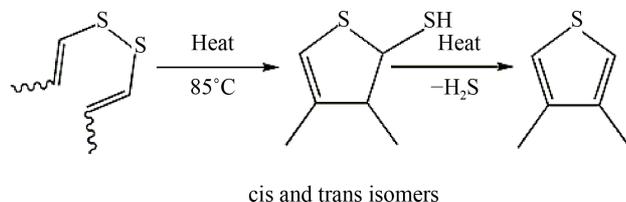


Figure 6. Formation of *cis*-, *trans*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene and 3,4-Dimethyl-2,3-dihydrothiophene from bis(1-propenyl)disulfide during heating process.

and GC-O could be used to investigate the characteristic aroma components present in TS. A 65 μm DVB/PDMS fibre was found to be an appropriate fibre for analyzing the volatiles in TS, and the optimal extraction conditions were 40°C for 30 min. Under these conditions, 56 peaks were separated and 53 compounds in TS volatiles were identified. Among them, sulfur compounds and terpenes accounted for a large proportion of the TS extract (42.146%, 55.984%, respectively). GC-O runs revealed the odor profiles of the extracted compounds. A total of 26 compounds were considered to be “aroma-active” and their odor intensities were evaluated. However, only a few of these compounds were found to contribute to the unique aroma of TS. Based on the data presented in this study, two compounds, *cis* and *trans* isomers of 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene appeared to determine the aroma of TS. This is the first report to confirm the identity of the key volatile compounds in TS.

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