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Original Research Article

Headspace Solid-Phase Microextraction Coupled with Gas Chromatography-Mass Spectrometric Analysis of Volatile Components of Raw and Stir-Fried Fruit of *C. Pinnatifida* (FCP)

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Abstract

Purpose: *To investigate the change of volatile components associated with odor of C. Pinnatifida (FCP) fruit and its stir-fried forms.*
Methods: *FCP fruit was stir-fried and monitored by an online-type and non-contact temperature measurement system (ONTMS). Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) was used to analyze the volatile composition of raw FCP and its various stir-fried forms.*
Results: *The color of FCP turned darker with the stir-frying process. In all, 47 volatile compounds with contents > 1 % were identified. The major volatile components were methyl acetate (4.40 %), n-hexane (2.90 %), 2-methyl-furan (1.80 %), 3-methyl-butyraldehyde (3.64 %), hexanal (2.08 %), furaldehyde (5.77 %), and D-limonene (7.99 %) in raw FCP. Following stir-frying, the contents of furaldehyde, 5-methyl-furaldehyde, methyl acetate, 2-methyl-butyraldehyde, D-limonene and 2-methyl-furaldehyde were altered significantly, which might have resulted in odor changes.*
Conclusion: *HS-SPME coupled with GC-MS is a rapid and eco-friendly method with the potential to analyze volatile compounds in raw and processed FCP.*

Keywords: *Crataegus pinnatifida, Stir-frying, Online-type and non-contact temperature measurement system, Headspace solid-phase microextraction, Volatile components, Odor*

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INTRODUCTION

Crataegus pinnatifida (Hawthorn) belonging to the family of Rosaceae includes *C. pinnatifida* Bge. var. major N.E.Br. and *C. pinnatifida* Bge. The fruit of *C. pinnatifida* (FCP) is a valuable and important traditional Chinese medicine and natural food, mainly distributed in the north of China [1]. It was first documented in Compendium of Materia Medica (*bencaogangmu* in Chinese) as an effective agent for treating dyspepsia and blood stasis [2]. Furthermore, FCP currently is commonly used to treat various diseases in Chinese folk medicine, including digestive disorders, blood stasis, cardiodynia, and hemafecia [1,2]. Previous phytochemical researches demonstrated that there were plenty of flavonoids, phenolic compounds, sugars,

sugar alcohols, organic acids, and terpenes in FCP [1,3]. In addition, modern pharmacological researches have demonstrated that FCP possesses significant promotive effects on digestion [4], lipid regulative and anti-atherosclerosis effects [5,6], anti-hypertensive effects [7], antioxidant [8] and antibacterial effects [9].

Based on the theory of traditional Chinese medicine (TCM) system, FCP should be processed before clinical application, and the processed products of FCP mainly include raw hawthorn and charred hawthorn [2,10]. Interestingly, it is proved that charred hawthorn possessed better therapeutic effect than raw hawthorn in treating digestive disorders [11]. Furthermore, the odor of the processed products of hawthorn is obviously different, and it has been suggested that odor change is one of the explanations for the different therapeutic effects. However, the link between changes in chemical composition and odor change has not been established.

In recent years, headspace solid phase micro-extraction (HS-SPME) has gained wide acceptance as an effective analytical technique for a wide variety of materials, including foods and drinks [12], especially for the aromatic materials. HS-SPME analytical method is eco-friendly and rapid compared with traditional analytical techniques, and it is performed by exposing a fiber coated with polymers to the headspace of samples without any solvent. The fiber selection is often based on the principle of "like dissolves like" [13].

The aim of the present study was to investigate variations in volatile profiles of raw FCP and its stir-fried forms using HS-SPME coupled with GC-MS.

EXPERIMENTAL

Materials

FCP was purchased from the *Hehuachi* market of traditional Chinese medicine, and was identified by Professor Min Li (College of Pharmacy, Chengdu University of Traditional Chinese Medicine) and the voucher specimen (Voucher No. 2012010) deposited at the Herbarium Centre of Pharmacognosy, Chengdu University of Traditional Chinese Medicine, Chengdu, China. An online and non-contact temperature measurement system (ONTMS), invented by our research team, used to monitor temperature during the stir-frying process (Table 1) [14].

Table 1: Stir-frying process conditions for FCP

NO.	Time (min)	Temperature (^{o}C)	Yield (%)
S_0	0		100
S_1	2	370 ± 30	99
S_2	4	380 ± 30	96
S_3	6	375 ± 30	96
S_4	8	360 ± 30	93
S_5	10	360 ± 30	92
S_6	12	400 ± 30	90

HS-SPME analysis

Approximately 3 g of pulverized sample, accurately weighed, was introduced into 20-mL headspace vial. The fibre was coated with 50/30 µm of Divinylbenzene-Carboxen-Polymethylsiloxane (DVB/CAR/PDMS), which is used for the absorption of volatile compounds. The sample was maintained at 70 ^{o}C for 20 min. During the sampling time, the sample was stirred at constant speed of 250 rpm. Following headspace extraction, SPME fibers were injected into the GC and remained in the GC inlet for 3 min.

GC-MS analysis

Volatile analysis was performed on an Agilent 7890A gas chromatograph coupled with a 5975C mass selective detector (Agilent Technologies, USA). Compounds were separated on a DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies). Injector temperature was 250 ^{o}C, and the split ratio was 1:1. Nitrogen of high-purity (99.999 %) was used as the carrier gas at a flow rate of 1 mL/min. The GC oven temperature was programmed as follows: 40 ^{o}C for 5 min, 5 ^{o}C/min to 215 ^{o}C. The interface temperature was 280 ^{o}C, and the quadrupole temperature was set at 150 ^{o}C. The mass spectrometer was fitted with an EI^{+} source operated at 70 eV with a source temperature of 250 ^{o}C, and mass spectra were recorded in the range of m/z 20 - 400 amu in the full scan acquisition mode. The electron multiplier voltage was 500 V.

Data analysis

In the present study, volatile compounds were identified by comparing the mass spectra of the analytes with those of authentic standards from the NIST database with a resemblance > 85 %. In addition, the compounds were analyzed based on 2 criteria: (1) the possibility index was used to confirm the identified constituents with a value >

750, (2) peak area relative to total peak areas > 1 % (i.e., amount of individual components expressed as % area of total peak areas).

RESULTS

Stir-fried FCP monitored by ONTMS

Figure 1 shows stir-fried FCP monitored by ONTMS in different processing time. The surface color of raw FCP is red and its pulpa brown or light brown. The color of stir-fried FCP turns darker gradually with the processing.

Volatile compounds identified by HS-SPME/GC-MS

The typical total ion chromatograms of the HS-SPME/GC–MS are shown in Figure 2. The results of the HS-SPME/GC–MS analysis are summarized in Table 2.

Volatile composition of raw FCP

In all, 20 volatiles were identified in raw FCP, including 13 carbonyls, and 7 additional volatiles. The major components identified in the HS-SPME extract of raw FCP were methyl acetate (4.40 %), n-hexane (2.90 %), 2-methyl-furan (1.80 %), 3-methyl-butyraldehyde (3.64 %), hexanal (2.08 %), furaldehyde (5.77 %), and D-limonene (7.99 %). Amongst these compounds, D-limonene possessed the highest content.

Changes in volatile compounds during the stir-frying process

HS-SPME/GC-MS indicated that a range of aldehydes, ketones, alcohols, aromatic hydrocarbons, furans and oxazoles were formed during the processing of stir-frying. To investigate how the contents of volatile compounds changed in FCP during the stir-frying process, the 6 main compounds (Figure 3) with a content > 2 % (relative percentage content) were selected, including furaldehyde, 5-methyl-furaldehyde, methyl acetate, 2-methyl-butyraldehyde, D-limonene and 2-methyl-furaldehyde. In addition, these 6 target compounds were selected as characteristic compounds of the stir-frying process, which might be the main contributors to odor change of FCP.

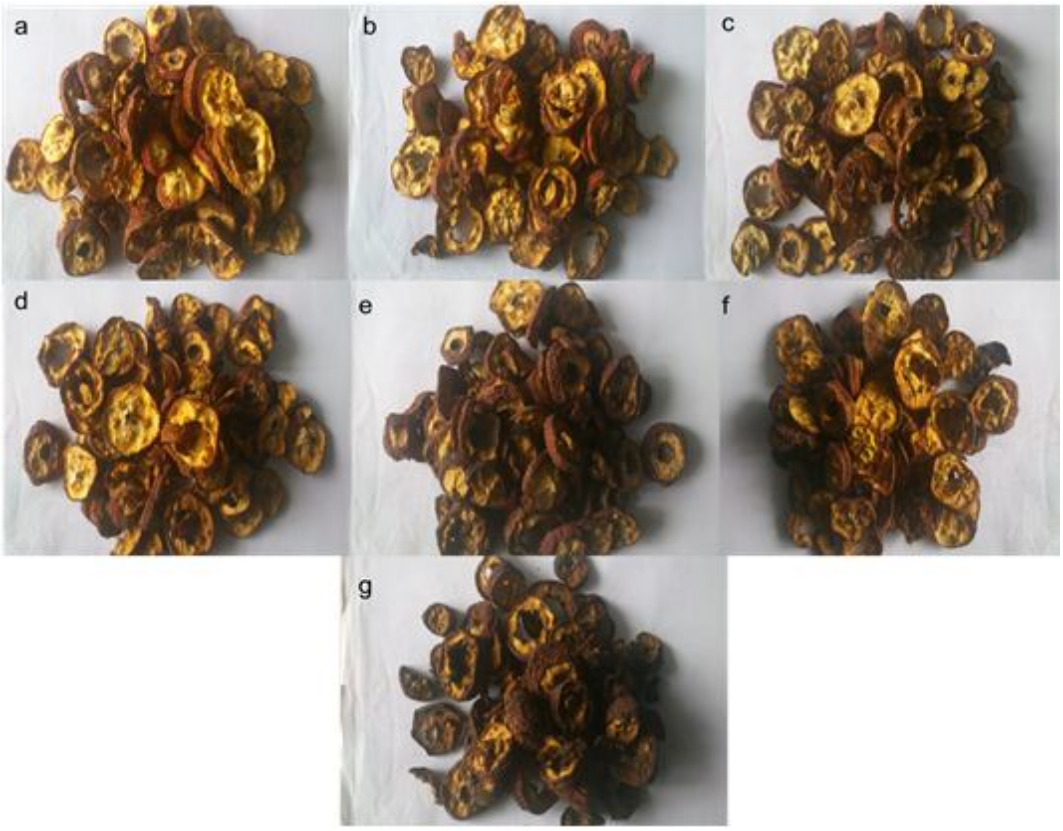

Figure 1: Stir-fried samples monitored by ONTMS collected at 0 min (a), 2 min (b), 4 min (c), 6 min (d), 8 min (e), 10 min (f) and 12 min (g), respectively

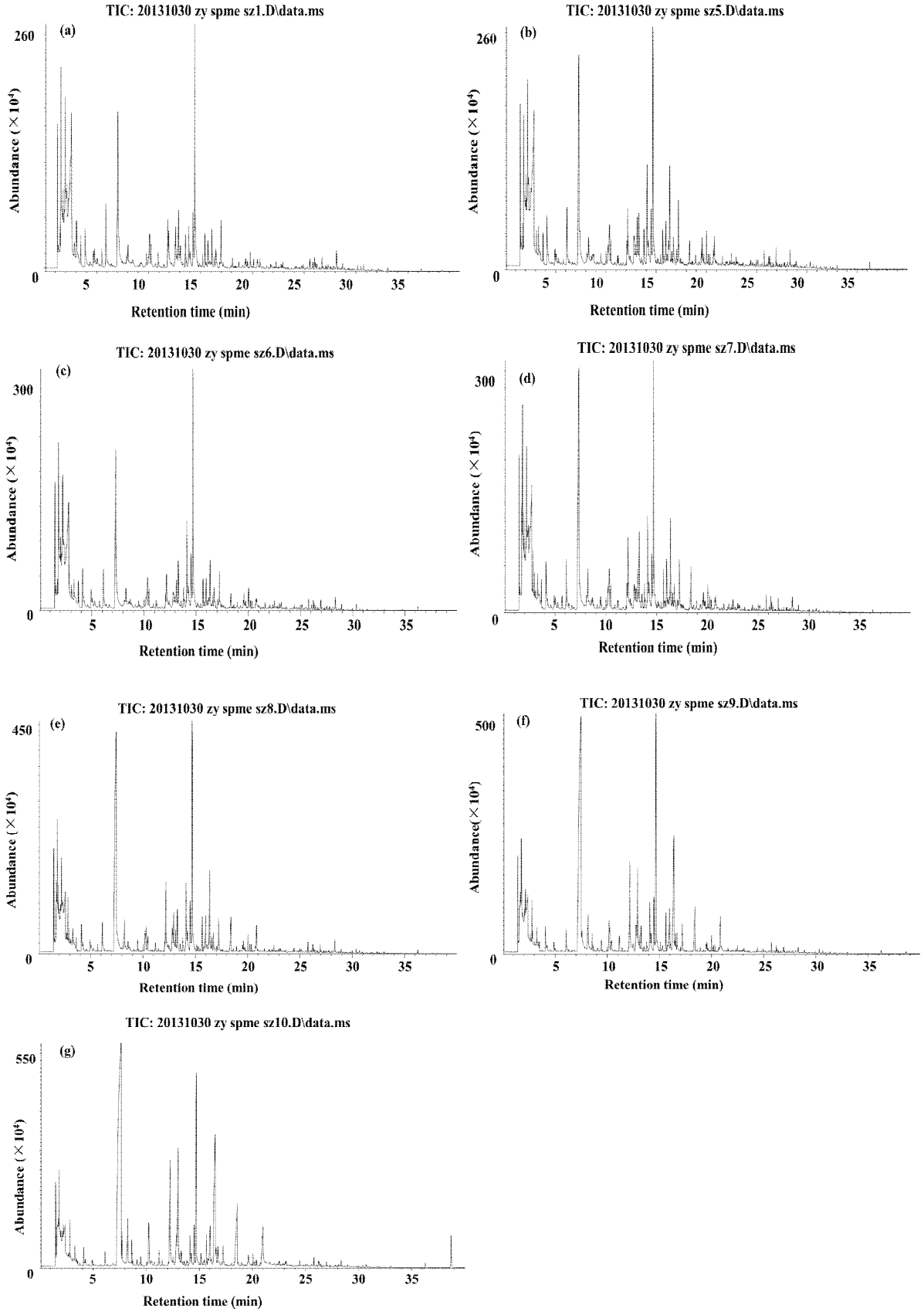

Figure 2: GC-MS total ion chromatogram of volatile components of the raw and processed products of FCP. a-g represented the raw FCP and its processed products stir-fried 2, 4, 6, 8, 10, 12 min, respectively

Table 2: Chemical composition analysis of the volatile constituents of the stir-frying process of FCP

RT (min)	Compound	Possibility	Relative contents (%)						
			Raw	2min	4min	6min	8min	10min	12min
1.712	Acetone	948	--	--	--	--	--	1.38	--
1.745	formic acid	933	--	--	--	--	--	1.41	--
1.797	borane-methyl sulfide complex	960	1.91	1.93	1.40	1.06	0.39	--	--
1.806	dimethyl sulfide	952	--	--	--	4.00	2.67	--	--
1.823	methyl acetate	926	4.40	4.21	2.93	2.67	2.06	1.39	--
2.173	n-hexane	907	2.90	--	2.36	--	--	--	--
2.199	2-methyl-furan	933	1.80	1.22	1.36	0.88	0.26	--	--
2.224	2,3-dimethyl-1-butene	726	--	2.22	--	1.88	--	--	--
2.233	2-methyl-octyl ester-2-propenoic acid	677	--	--	--	--	1.26	--	--
2.524	4-methyl-1,3-pentadiene	938	--	--	--	1.6	1.39	--	--
2.644	2,3-dihydro-furan	821	--	--	--	2.30	--	--	--
2.652	2-butenal	844	1.88	1.71	--	--	--	--	--
2.712	3-methyl-butyraldehyde	800	3.64	3.71	3.91	4.03	--	--	--
2.823	2-methyl-butanal	900	1.84	1.27	--	--	--	--	--
3.293	Pentanal	910	1.35	--	--	--	--	--	--
4.114	(E)-3-penten-2-one	931	1.54	1.47	1.36	1.13	--	--	--
4.131	1-[(1-oxo-2-propenyl)oxy]-pyrrolidine-2,5-dione	724	--	--	--	1.13	--	--	--
4.14	1-penten-3-one	626	1.94	--	--	--	--	--	--
6.114	Hexanal	905	2.08	1.41	1.4	1.30	--	--	--
7.226	5-methyl-2(3H)-furanone	650	--	1.30	--	--	--	--	--
7.261	2,4-dihydro-5-methyl-3H-pyrazol-3-one	699	--	--	--	3.30	--	--	--
7.303	5-methyl-3-isoxazolamine	665	--	--	--	--	5.32	--	--
7.321	Furaldehyde	933	5.77	6.81	8.45	11.23	18.95	18.52	23.87
7.525	2-oxo-methyl butyrate	750	--	--	--	--	--	18.27	--
8.209	2-furanmethanol	955	--	--	--	--	--	1.02	1.15
10.202	2(5H)-furanone	890	--	--	--	--	--	--	1.43
10.227	1-(2-furanyl)-ethanone	953	--	--	--	--	--	--	1.43
10.338	Butyrolactone	904	1.27	1.36	1.37	1.51	--	--	--
12.083	(Z)-2-heptenal	904	1.09	--	--	--	--	--	--
12.16	5-methyl-2-furancarboxaldehyde	954	1.50	1.71	1.47	2.01	2.37	2.30	3.09
12.835	1-decylene-3-one	805	1.75	--	--	--	--	--	--
12.852	1,2-glycol diacetate	615	--	--	--	--	--	--	1.07
12.937	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	874	--	--	--	--	1.16	2.07	3.43
13.014	D-alanine, N-butoxycarbonyl-dodecyl ester	702	--	--	--	--	--	--	3.37
13.117	6-methyl-5-heptene-2-one	933	1.60	1.11	--	--	--	--	--
13.271	2H-pyran-2,6(3H)-dione	797	--	1.02	--	1.51	1.06	--	--
13.279	2,2,4,6,6-pentamethyl-heptane	900	--	1.02	1.27	1.50	1.06	--	--
14.126	ethyl acetate	711	--	--	2.82	--	--	--	--
14.502	O-cymene	956	1.52	1.09	1.52	1.14	--	1.03	--
14.511	1-methyl-3-(1-methylethyl)-benzene	943	--	--	--	--	1.38	--	--
14.682	D-limonene	919	7.99	7.47	7.13	5.73	5.61	5.11	4.13
15.973	dihydro-3-methylene-5-methyl-2-furanone	756	--	--	--	1.11	1.11	1.13	1.24
16.357	2,5-furandicarboxaldehyde	837	--	--	--	--	--	--	1.12
16.408	3-furancarboxylic acid methyl ester	901	--	2.03	1.72	1.82	2.56	3.53	5.61
17.221	Nonanal	943	1.69	1.31	1.10	1.07	--	--	--
18.375	2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	892	--	--	--	1.13	1.32	1.51	3.10
20.897	5-hydroxymethylfurfural	889	--	--	--	--	--	1.12	2.02

RT = Retention time; "--" means the contents < 1 %

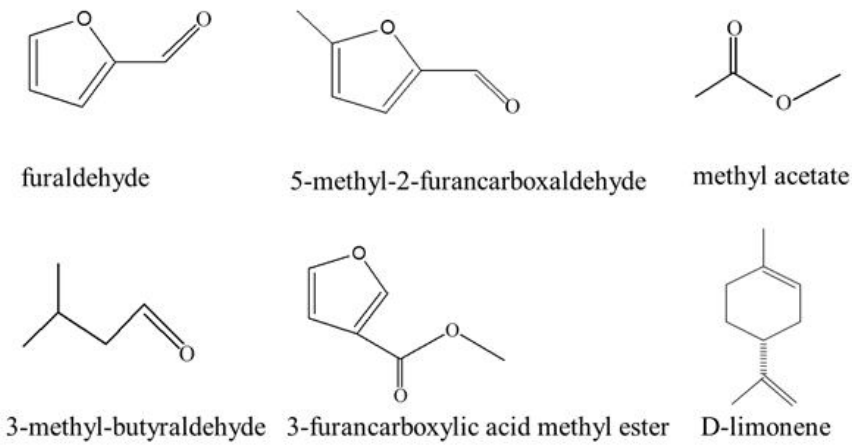

Figure 3: Chemical structures of the main constituents of volatile composition

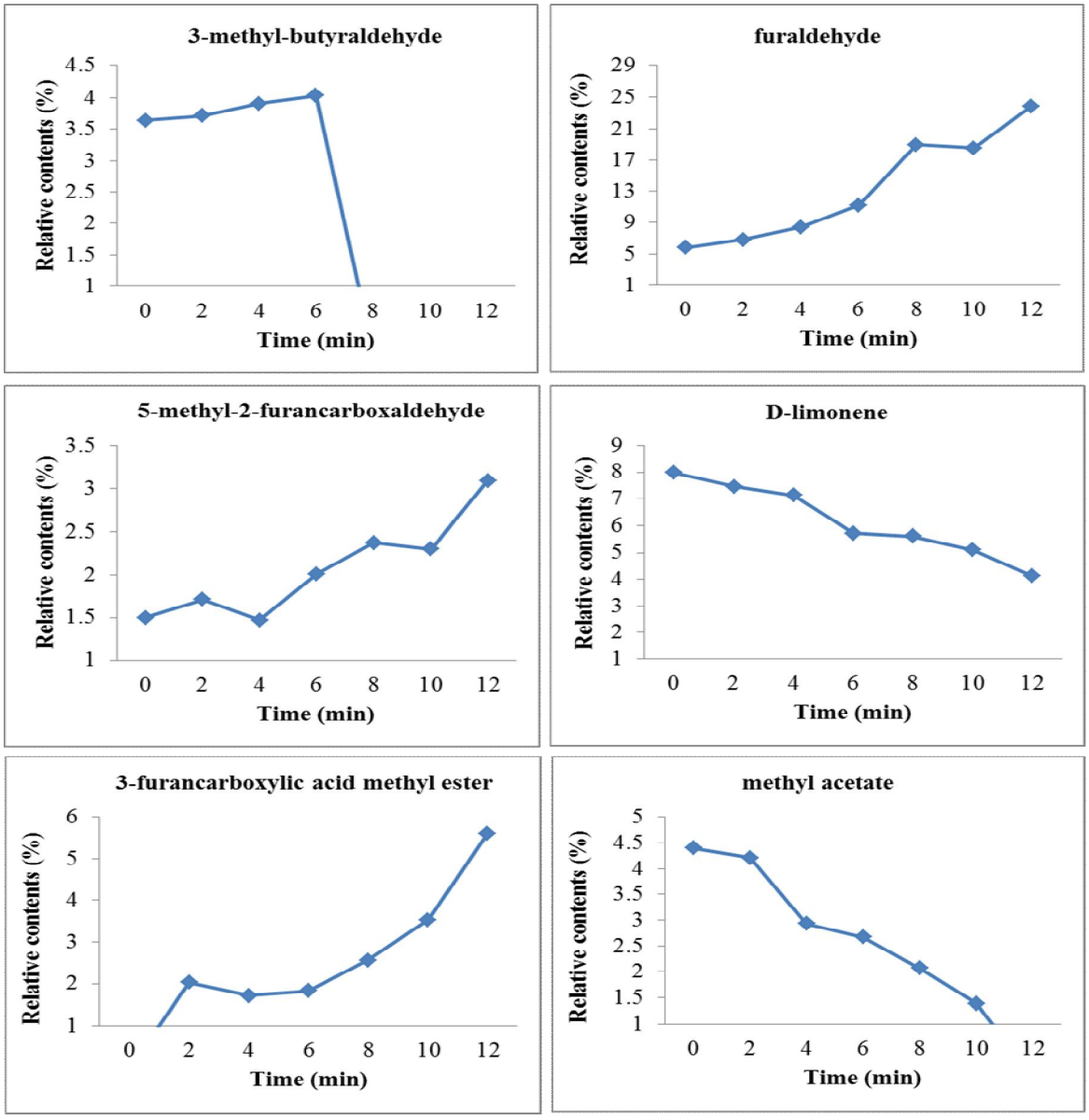

Figure 4: Contents change of the main constituents of volatile composition during the stir-frying process

In the present investigation, the results showed that contents of 5-methyl-furaldehyde, furaldehyde, 3-furancarboxylic acid methyl ester increased gradually during the processing of FCP, whereas the contents of D-limonene, and methyl acetate decreased. For the contents of 3-methylbutyraldehyde, it increased with the processing of stir-frying, then decreased (Figure 4).

DISCUSSION

The ONTMS provided an effective method to monitor the dynamic temperature and through this method we get ideal stir-fried FCP products. Aroma is one of the most important indexes to evaluate quality of aromatic foods [15] and Chinese herbal medicines [16]. The quality of aromatic medicines was often evaluated by GC or GC-MS method using essential oil extracted by steam distillation. Compared to this method, GC-MS following HS-SPME is a solvent-free, rapid and convenient method for the pre-concentration, increasingly used for analysis of the volatile compounds from aromatic herbal medicines. To our best knowledge, the present investigation is the first work regarding the odor and chemical composition of the raw FCP and its stir-fried products. Our results suggest that the odor changes of different processed FCP might be mainly correlated with 5-methyl-furaldehyde, furaldehyde, 3-furancarboxylic acid methyl ester, 3-methyl-butanal, D-limonene and methyl acetate.

Maillard reaction is known as amino-carbonyl reaction or non-enzymatic browning reaction [17], which occurs during food processing, storage and produces a large number of Maillard reaction products, including non-volatile colored compounds of intermediate molecular mass, volatile compounds of low molecular mass, and complex brown substances of high molecular weight. These volatile compounds were mainly divided into 4 types, including N-containing heterocyclic compounds, cyclic ketene-alcohol compounds, single-carbonyl compounds and multi-carbonyl compounds [18], which were mainly contributed to odor, flavor and color of foods. Previous investigations have demonstrated that the 5-hydroxymethyl furfural and its derivatives are the important and common intermediate products of the processing of traditional Chinese medicine mainly via Maillard reaction [19]. According to the present results, contents of 5-hydroxymethylfurfural and 5-methyl-furaldehyde were increased gradually during the processing, indicating that the odor changes might be related to the Maillard reaction. In addition, FCP is rich in sugar and amino acids in the aspect of chemical composition based on previous phytochemical investigations, which creates conditions for Maillard reaction.

CONCLUSION

The odor-active compounds and contents of volatile profiles of stir-fried FCP depend largely on stir-frying time. HS-SPME coupled with GC-MS is a rapid and eco-friendly method to analyze volatile profiles of different stir-fried FCP. It is speculated that the change of these volatile compounds and odor may be caused by the Maillard reaction.

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