Study of the flavor volatiles in double-fried pork: Identification and analysis of their changes during frozen storage using headspace solid-phase microextraction and gas chromatography-mass spectrometry.

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Abstract

The flavor volatiles present in double-fried pork and their changes during frozen storage were investigated for the first time using head space-solid phase micro extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) methods. We identified total 91 types of volatiles including eighteen heterocyclic compounds (6.9%), fifteen alcohols (11.4%), thirteen aldehydes (51.2%), twelve esters (7.7%), eleven hydrocarbons (2.3%), seven sulfur compounds (9.4%), six ketones (7.0%), and four acids (1.4%). Both the content and the types of heterocyclics and esters significantly increased post cooking. Principal component analysis showed three principal components (PC) with the first one (PC1) representing majority of the flavor compounds (73.65%), identified as the characteristic flavors. We could distinguish between the samples with different frozen time from the distinct flavors: (i) 1-penten-3-ol, 3-methyl-1-butanol and octanal in one month frozen samples; (ii) nonanal, *trans*-2,4-decadienal, and phenylethyl alcohol in 2-3 months frozen samples, and (iii) hexanal and ethanol in the 5-6 months frozen samples.

Keywords: Double-fried pork, Volatile compounds, GC-MS analysis, frozen storage, PCA analysis.

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Introduction

Pork, an important nutritional ingredient in the human diet, is rich in high-quality protein, essential fatty acids, and some vital trace elements such as calcium, phosphorus, iron, and thiamin [1]. Double-fried cooking, a characteristic processing technique for pork in China, has also been popular all over the world in recent years. Flavor of double-fried pork is considered as the primary palatability factor and is shown to be of great importance. The number of flavor volatile compounds, identified in the extracts of livestock and poultry meat, is over one thousand; those include aldehydes, ketones, esters, ethers, alkanes, alkenes, alcohols, carboxylic acid, and some heterocyclic compounds (oxygen, nitrogen and sulfur) [2]. The flavor of pork is not only influenced by breed, age, gender, feed environment of animals, but also affected by the physical and chemical factors such as processing methods (cooking, baking, or fermentation process), storage conditions, or the addition of different pickled formulations and spices [3,4]. Many researchers have studied the flavor of pork processed by different methods, for example, Zeng et al. [5] investigated the compositions of flavor compounds of pork, boiled in clear soup or braised in soy sauce; Chen et al. [6] studied the aroma of pork slices cooked in fermented mustard greens; Du [7] determined the flavor of fermented pork, while Qin et al. [8] investigated the variations of aroma compounds in roasted Rongchang pork under different temperature conditions. However, little information available on the flavor compounds of pork, processed by double-fried cooking. Therefore, the objective of this study was to identify the flavor compounds in double-fried pork and to investigate the changes in these flavors during frozen storage. To the best of our knowledge, this is the first report, where the flavor volatiles present in double-fried pork were extracted using head space-solid phase micro extraction (HS-SPME) technique -a rapid, inexpensive, and solvent-free method for extracting volatile and semi-volatile [9].

Materials and Methods

Materials

Streaky pork, green onion, ginger, garlic and pepper were purchased from local market. Thick broad-bean sauce, flavor bean sauce and mushroom sauce were supported by PIXIAN Watereress Co., Ltd. (Sichuan, China), Chuannan Fermentation Co., Ltd. (Sichuan, China), and Foshan Haitian Flavoring and Food Co., Ltd. (Guangdong, China), respectively. Salt (food grade), white granulated sugar, and rice wine were purchased from Jiangsu Salt Industry Group Co., Ltd. (Jiangsu, China), Hangzhou Luzhibao Food Co., Ltd. (Zhejiang, China), and Huzhou Laoheng Fermentation Co., Ltd. (Zhejiang, China), respectively. Ajinomoto was purchased from Taitaile Food Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade.

Sample preparation

Figure 1 presents the flow chart of preparing double-fried pork. Following the preliminary practice of meat processing, water

Streaky pork ⇒ Precooking ⇒ Cooling ⇒
Cutting to slice ⇒ Double-cooking ⇒ Product

Figure 1: *The flow chart of the manufacture of double-fired pork.*

was boiled with powdered ginger and garlic, scallions, pepper, rice wine and salt for approximately 30 s. The streaky pork was rolled to 60-80% mature so that chopsticks can be inserted through into the pork, taken out to form "lamp socket" more easily, the key is to have a little meat and a little blood in the middle area), and cooled in the refrigerator for 3 min (while high temperature facilitates the separation of fat and lean meat, low temperature is not conducive for preparing pork slices). Finally, the cooked pork was cut down to fillet (~5 cm long, 3 cm wide and 0.2 cm thick). The double-fried cooking of pork was carried out in an induction cooker at 180°C for 3.5 min. A little vegetable oil was poured on the pre-heated pan (40% heating); when the oil temperature reached a certain degree (90°C), the precooked pork was put into the pan, and stir-fried for 1.5 min (the oil temperature was maintained at about 90°C by keeping the heating temperature at 180°C for 1.5 min. The pork fat curled up, forming "lamp socket". At this point, thick broad-bean sauce and flavor bean sauce were added and cooked for 1 min. Next, green onion, ginger, garlic, white granulated sugar, Ajinomoto, mushroom sauce and rice wine were added and again cooked for 1 min to obtain the final product of double-fried pork, which was stored at -20°C for further analysis.

Headspace solid-phase microextraction (HS-SPME) for the volatiles of double-fried pork

A 75 μm carboxen/poly (dimethylsiloxane) (CAR/PDMS) coated fiber (Supelco, Inc., Bellefonte, PA, USA) was used for the HS-SPME of the volatile compounds from the double-fried pork sample. Each pork sample (7 g) was cut, mashed and put into a 15 ml glass bottle. The glass bottle was then tightly capped with a silicon septum. The samples were equilibrated at 65°C for 10 min, and the volatile compounds were extracted at the same temperature (65°C) with stirring for 30 min in a multipurpose sampler with SPME capability (MPS2, Gerstel, Germany). Immediately after extraction, the fiber was inserted into the injection port of GC (gas chromatography) for 5 min at 250°C (in split mode) to desorb the analytes. All experiments were carried out in duplicate.

Gas chromatography-mass spectrometry (GC-MS) analysis for the volatiles of double-fried pork

Analysis of the flavor compounds of double-fried pork was performed on a 1200 L GC/MS-MS system (Finnigan Co., USA) equipped with a 1079 middle injector and a mass selective detector (MSD). The MSD was used to identify unknown compounds. The separation of volatile and semi-volatile compounds was performed using a fused silica capillary column (specification: 50 m × 0.32 mm i.d., coated with BPX-5 at 0.5 μm film thickness, SGE Ltd.). The temperature of both the injector and the detector was set at 250°C. Following was the GC program for identification: an oven temperature of 40°C held for first 2 min, then heating to 100°C (5°C/min) held for 0 min, and finally, heating to 250°C (10°C/min) held for 20 min. Helium was used as the carrier gas with a flow rate of 70 μl/min and a split ratio of 50 ml/min. Mass spectra were recorded in the centroid scan mode at an ionization voltage of 70 eV, an emission current of 50 μA for filament, and an ion source temperature of 200°C. Electron impact (EI) mass spectra were recorded in the 33-450 amu range with a scan time of 0.400 s. Volatiles were identified by comparing their mass spectra with the mass spectra from MS libraries (NIST/WILEY/REPLIB/MAINLIB) [10]. The volatiles were identified according to the matched-degree as well as the purity (>800). The relative percentage content of each compound was calculated by area normalization method. Principal component analysis (PCA) was performed to determine the respective contribution of various flavor compounds on the total aroma, and the synthesis score of principal components was calculated as follows:

$$PCn = fn \times \sqrt{\lambda_n} \quad (1)$$

$$PC = PC1 \times \frac{\lambda_1}{\lambda_1+\lambda_2+\ldots+\lambda_n} + PC2 \times \frac{\lambda_2}{\lambda_1+\lambda_2+\ldots+\lambda_n} + \ldots + PCn \times \frac{\lambda_n}{\lambda_1+\lambda_2+\ldots+\lambda_n} \quad (2),$$

Where *PCn* is the principal component *n*, fn is the score of *PCn*, λ_n is the characteristic value of *PCn*, and *PC* is the total score or synthesis score.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, Illinois USA). The results were analyzed by performing the analysis of variance (ANOVA) procedure. Correlation analysis was performed to determine the significant differences in the double-fried pork samples with different frozen storage times [11].

Results and Discussions

Tables 1 and 2 show that the amount of the main flavor compounds (such as aldehydes, hydrocarbons, and alcohols) is higher in raw pork than those in double-fried pork. However, we found that the relative amount of some trace volatiles (ketones, esters, acids, heterocyclics and sulfurs), especially the esters and heterocyclics, in double-fried pork increased significantly. Moreover, the types of the volatiles of esters, heterocyclics, sulfurs increased in pork after cooking, while other flavor substances reduced in the number of species. The largest changes in relative amounts occurred in the ester and heterocyclic contents. The relative content of esters dramatically increased from 0.21% to 7.74% with increase in the types from six to twelve, while the heterocyclic content increased from 0.36% to 6.87% with eighteen types. Furthermore, the aroma of boiled specimens was soft and downy to some extent, mainly exhibiting a flavor profile comprising of grass aroma, fat aroma and fruit aroma. On the other hand, the aroma of cooked specimens was nutty, butyric, fruity, and burnt. During the frozen storage processing, we did not find any significant changes in the relative contents and types of volatiles in double-fried pork. Among heterocyclic compounds, we found mainly furans, pyrazines and pyrroles in double-fried pork. Furans contribute grain fragrant and sweet smell to the overall aroma of double-fried pork. The furans, we identified in the double-fried pork, were mainly 2-amyl furan, 2-acetyl furan, furfural and furfuryl alcohol. Pyrazines, found in double-fried pork, are mainly alkyl pyrazines. It forms in the Strecker degradation, with dicarbonyl compounds and amino acids forming alpha amino ketones and alpha amino ketones through condensation and oxidation, forming pyrazine. The threshold of methyl pyrazine is relatively high (>1 × 10^{-6} g/g) with little impact on pork flavor. However, if one or more of the methyl group is replaced by the ethyl, the corresponding smell

Table 1: *Result of GC-MS analysis for the relative content (%) of volatile flavors of double-fried pork during cooking process and frozen storage.*

Number	Volatile compounds Aldehydes	A	B	C	D	E	F	G	H
1	Pentanal	2.500	2.251	2.006	2.265	2.132	2.238	2.284	2.216
2	Butanal, 3-methyl-	nd	2.926	3.168	2.899	3.322	2.879	2.934	2.949
3	Hexanal	46.665	37.632	37.159	37.717	38.001	37.829	37.776	37.883
4	2,4 Pentadienal	1.817	nd	nd	nd	nd	nd	nd	nd
5	Heptanal	2.401	0.691	0.736	0.668	0.753	0.726	0.772	0.719
6	2-Hexenal	0.063	nd	nd	nd	nd	nd	nd	nd
7	Octanal	1.848	0.631	0.649	0.592	0.561	0.662	0.661	0.672
8	2-Heptenal, (Z)-	0.589	nd	nd	nd	nd	nd	nd	nd
9	Nonanal	3.421	2.252	2.139	2.366	2.347	2.402	2.306	2.282
10	2 Octenal	0.466	0.298	0.269	0.113	0.109	0.101	0.097	0.088
11	Benzaldehyde	nd	1.292	1.269	1.402	1.438	1.532	1.623	1.701
12	2-Nonenal, (E)-	nd	0.099	nd	nd	nd	nd	nd	nd
13	Benzeneacetaldehyde	nd	1.816	1.944	1.809	1.854	1.914	1.918	1.859
14	Decanal	0.052	nd	nd	nd	nd	nd	nd	nd
15	Octadecanal	0.016	nd	nd	nd	nd	nd	nd	nd
16	d-Myrtenal	0.049	nd	nd	nd	nd	nd	nd	nd
17	Z-Citral	0.377	0.501	0.543	0.467	0.475	0.502	0.484	0.457
18	E-Citral	0.415	0.739	0.792	0.673	0.657	0.715	0.651	0.624
19	2,4-Decadienal, (E,E)	0.059	0.159	0.164	0.179	0.168	0.172	0.165	0.159
	Alcohols								
20	Ethanol	0.758	2.02	2.11	2.36	2.85	3.754	4.265	4.195
21	Carveol, dihydro-, cis-	nd	1.397	1.379	1.210	1.143	1.154	1.162	1.166
22	2-Propen-1-ol	0.043	1.381	1.352	1.326	1.039	1.034	1.042	1.025
23	1-Butanol	0.205	0.182	0.237	0.255	0.243	0.241	0.237	0.319
24	1-Penten-3-ol	0.117	0.231	0.226	0.168	0.124	0.154	0.169	0.176
25	1,8-Cineole	1.589	0.388	0.388	0.343	0.288	0.276	0.253	0.296
26	1-Butanol, 3-methyl-	nd	0.701	0.679	0.519	0.466	0.419	0.503	0.685
27	3-Buten-1-ol, 3-methyl-	0.063	nd	nd	nd	nd	nd	nd	nd
28	1-Pentanol	4.492	1.371	1.319	1.299	1.581	1.657	1.391	1.310
29	1-Hexanol	0.979	0.658	0.450	0.524	0.381	0.2	0.148	0.138
30	1-Octen-3-ol	2.938	1.932	1.858	1.881	2.001	1.944	1.848	1.907
31	1-Heptanol	0.688	nd	nd	nd	nd	nd	nd	nd
32	Thujyl alcohol	0.156	nd	nd	nd	nd	nd	nd	nd
33	2-Decanol	0.034	nd	nd	nd	nd	nd	nd	nd
34	Cyclohexanol, 1-methyl-4-(1-methyletheny	0.126	nd	nd	nd	nd	nd	nd	nd
35	p-Menth-8-en-1-ol, stereoisomer	0.084	nd	nd	nd	nd	nd	nd	nd
36	2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-, trans-	0.098	nd	nd	nd	nd	nd	nd	nd
37	1-Hexanol, 2-ethyl-	0.067	nd	nd	nd	nd	nd	nd	nd
38	Linalool	0.207	0.247	0.304	0.225	0.263	0.317	0.266	0.266
39	1-Octanol	0.506	0.152	0.141	0.134	0.128	0.120	0.117	0.165
40	2,3-Butanediol	0.093	nd	nd	nd	nd	nd	nd	nd
41	Terpinen-4-ol	0.373	0.210	0.170	0.054	0.093	0.102	0.086	0.083
42	2-Octen-1-ol-(E)	0.418	0.189	0.138	0.105	0.091	0.085	0.064	0.058
43	alpha-Terpineol	0.204	nd	0.146	nd	nd	nd	nd	nd
44	Borneol	0.115	nd	nd	nd	nd	0.07	nd	nd
45	Nerol	0.225	nd	nd	nd	nd	nd	nd	nd
46	Geraniol	0.329	nd	nd	nd	nd	nd	nd	nd
47	Phenylethyl Alcohol	0.130	0.353	0.322	1.095	0.827	0.156	0.126	0.130
48	Beta-Fenchyl alcohol	nd	nd	nd	nd	0.09	nd	nd	nd
	Hydrocarbons								
49	Octane	0.051	nd	nd	nd	nd	nd	nd	nd
50	Heptane, 2,4-dimethyl-	0.057	nd	nd	nd	nd	nd	nd	nd
51	Decane	0.031	nd	nd	nd	nd	nd	nd	nd
52	Undecane	nd	nd	nd	nd	nd	nd	nd	nd
53	Tridecane	nd	nd	nd	nd	nd	nd	nd	0.094
54	Toluene	3.989	0.405	0.427	0.526	0.581	0.665	0.956	0.840
55	Camphene	0.222	0.826	1.073	0.673	0.391	0.146	0.134	0.211
56	a-Curcumene	nd	nd	0.157	nd	nd	nd	nd	nd

57	Styrene	nd	0.112	0.107	0.102	0.096	0.078	0.056	0.08
58	Camphene hydrate	0.097	nd	nd	nd	nd	nd	nd	nd
59	Sabinene	0.135	nd	nd	nd	nd	nd	nd	nd
60	Benzene, ethyl-	0.123	0.068	0.070	0.065	0.058	0.052	0.064	0.054
61	p-Xylene	0.076	0.078	0.074	0.070	0.071	0.066	0.062	0.068
62	beta.-Pinene	0.113	nd	nd	nd	nd	nd	nd	nd
63	alpha.-Copaene	0.083	nd	nd	nd	nd	nd	nd	nd
64	l-Phellandrene	0.030	0.171	0.162	0.167	0.156	0.155	0.168	0.152
65	beta.-Phellandrene	nd	0.825	0.731	0.538	0.533	0.156	0.217	0.277
66	beta.-Myrcene	0.257	0.700	0.136	0.141	0.098	0.074	0.068	0.073
67	1,3,6-Octatriene, 3,7-dimethyl-, (Z)	nd	0.143	nd	nd	nd	nd	nd	nd
68	trans-Caryophyllene	nd	0.161	nd	nd	nd	nd	nd	nd
69	Cyclohexene, 1-methyl-4-(1-methylethylid	0.089	nd	nd	nd	nd	nd	nd	nd
70	.alpha.-Terpinene	0.056	nd	nd	nd	nd	nd	nd	nd
71	l-Limonene	0.312	0.541	0.425	0.494	0.455	0.502	0.607	0.509
72	gamma.-Terpinene	0.083	nd	nd	nd	nd	nd	nd	nd
73	Benzene, 1-methyl-3-(1-methylethyl)-	0.068	nd	nd	nd	nd	nd	nd	nd
74	o-Cymene	0.089	nd	nd	nd	nd	nd	nd	nd
75	Cyclopentane, (2-methylpropylidene)-	0.100	nd	nd	nd	nd	nd	nd	nd
76	Limonene oxide	0.135	nd	nd	nd	nd	nd	nd	nd
77	beta.-Bisabolene	0.252	nd	nd	nd	nd	nd	nd	nd
78	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-	0.159	nd	nd	nd	nd	nd	nd	nd
79	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-me	0.252	nd	nd	nd	nd	nd	nd	nd
	Ketones								
80	Acetone	nd	3.704	3.682	3.655	3.469	3.124	2.766	2.183
81	2-Propanone, 1-hydroxy-	nd	0.256	0.283	0.246	0.275	0.297	0.313	0.355
82	2-Heptanone	0.176	nd	nd	nd	nd	nd	nd	nd
83	2-Butanone, 3-hydroxy-	0.251	0.172	0.189	0.197	0.175	0.179	0.179	0.231
84	1-Octen-3-one	0.077	nd	nd	nd	nd	nd	nd	nd
85	4-Octen-3-one	0.083	nd	nd	nd	nd	nd	nd	nd
86	2,3-Octanedione	2.928	2.529	2.595	2.665	2.732	2.714	2.803	2.842
87	5-Hepten-2-one, 6-methyl-	0.204	0.205	0.232	0.251	0.379	0.401	0.366	0.419
88	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimet	0.075	nd	nd	nd	nd	nd	nd	nd
89	p-menth-1-en-3-one	0.303	nd	nd	nd	nd	nd	nd	nd
90	Xanthoxylin	0.353	nd	nd	nd	nd	nd	nd	nd
91	Ethanone, 1-(2-hydroxy-4,6-dimethoxyphen	nd	0.117	nd	0.107	nd	nd	nd	nd
92	2-Cyclohexen-1-one, 2-methyl-5-(1-methyl)	nd	nd	nd	nd	0.335	nd	nd	nd
93	2-Propanone, 1-(acetyloxy)-	nd	nd	nd	nd	nd	nd	0.087	0.095
	Esters								
94	Formic acid, 2-propenyl ester	nd	0.733	nd	nd	nd	nd	nd	nd
95	Methyl laurate	nd	nd	nd	nd	nd	nd	nd	0.097
96	2,2-Dimethyl-6-oxoheptanoic acid methyl ester	nd	nd	nd	nd	nd	nd	nd	0.077
97	1-Butanol, 3-methyl-, acetate	nd	0.071	0.064	0.060	0.051	0.045	0.043	0.047
98	Hexanoic acid, ethyl ester	nd	0.110	0.201	0.168	0.309	0.351	0.269	0.252
99	Propanoic acid, 2-hydroxy-,ethyl ester	0.069	0.172	2.045	2.407	2.284	2.248	2.404	2.488
100	Octanoic acid, ethyl ester	nd	0.065	nd	nd	nd	nd	nd	nd
101	Linalyl acetate	nd	2.687	2.428	2.401	2.359	2.363	2.454	2.428
102	Propanoic acid, 2-methyl-, 3,7-dimethyl-	nd	0.713	0.72	0.693	0.801	0.776	0.719	0.721
103	Benzoic acid, methyl ester	nd	0.159	0.242	0.152	0.169	0.172	0.164	0.159
104	2(3H)-Furanone, dihydro-	nd	0.191	0.382	0.231	0.465	0.325	0.372	0.368
105	Benzoic acid, ethyl ester	nd	2.552	2.683	2.646	2.776	2.808	2.683	2.641
106	Benzeneacetic acid, ethyl ester	nd	0.062	0.079	0.081	0.073	0.068	0.074	0.072
107	Butanedioic acid, diethyl ester	nd	0.221	0.219	0.223	0.213	0.216	0.215	0.218
108	Acetic acid, butyl ester	0.039	nd	nd	nd	nd	nd	nd	nd

109	Acetic acid, cyano-, 2-ethylhexyl ester	0.043	nd	nd	nd	nd	nd	nd	nd
110	Glycerol 1,2-diacetate	0.038	nd	nd	nd	nd	nd	nd	nd
111	(3S)-1,3-Diacetoxy Butane(impure)	0.015	nd	nd	nd	nd	nd	nd	nd
112	3,6-Octadecadiynoic acid, methyl ester	0.008	nd	nd	nd	nd	nd	nd	nd
	Acids								
113	Acetic acid	0.126	0.861	0.898	0.935	0.987	1.047	1.095	1.109
114	Butanoic acid	nd	0.201	0.198	0.192	0.183	0.187	0.184	0.179
115	Pentanoic acid	0.104	nd	nd	nd	nd	nd	nd	nd
116	Hexanoic acid	0.231	0.173	0.162	0.168	0.157	0.172	0.174	0.168
117	Octanoic acid	0.036	nd	nd	nd	nd	nd	nd	nd
118	Nonanoic acid	0.020	nd	nd	nd	nd	nd	nd	nd
119	Decanoic acid	0.022	nd	nd	nd	nd	nd	nd	nd
120	Hexadecanoic acid	0.087	0.198	0.186	0.191	0.182	0.186	0.197	0.198
	Heterocyclic compounds								
121	Furan, 2-ethyl-	nd	0.558	0.553	0.562	0.545	0.554	0.531	0.488
122	2-Furancarboxaldehyde	nd	0.536	0.704	0.806	0.903	0.929	0.943	0.901
123	2-Furanmethanol	nd	0.817	0.831	0.811	0.904	0.907	0.921	0.935
124	2-Furanmethanol, 5-methyl-	nd	0.077	0.072	0.079	0.071	0.068	0.071	0.057
125	2-Furancarboxaldehyde, 5-methyl-	nd	0.101	0.122	0.137	0.148	0.189	0.191	0.194
126	Furan, 2-pentyl-	0.357	0.191	0.198	0.193	0.199	0.261	0.272	0.277
127	Pyrazine, methyl-	nd	0.669	0.648	0.633	0.679	0.646	0.668	0.714
128	Pyrazine, ethyl-	nd	0.098	0.086	0.091	0.096	0.092	0.085	0.088
129	2,5-dimethyl pyrazine	nd	1.220	1.201	1.325	1.287	1.352	1.393	1.294
130	Pyrazine, 2,6-dimethyl-	nd	0.591	0.536	0.573	0.552	0.529	0.598	0.532
131	Pyrazine, 2-ethyl-3-methyl-	nd	0.280	0.268	0.275	0.272	0.261	0.246	0.237
132	Pyrazine, 2-ethyl-5-methyl-	nd	0.073	0.071	0.062	0.060	0.057	0.053	0.051
133	Pyrazine, 2-ethyl-6-methyl-	nd	0.078	0.072	0.069	0.068	0.061	0.044	0.057
134	Pyrazine, 3-ethyl-2,5-dimethyl-	nd	0.201	0.193	0.189	0.191	0.186	0.173	0.182
135	Pyrazine, trimethyl-	nd	0.324	0.317	0.319	0.338	0.305	0.296	0.291
136	Ethanone, 1-(1H-pyrrol-2-yl)-	nd	0.554	0.518	0.505	0.548	0.402	0.432	0.472
137	Ethanone, 1-(2-furanyl)-	nd	0.316	0.324	0.341	0.295	0.268	0.226	0.228
138	1H-Pyrrole, 1-methyl-	nd	0.189	0.184	0.176	0.175	0.162	0.154	0.151
	Sulphur-Containing Compounds								
139	2-Propen-1-thiol	5.637	4.15	4.291	4.186	3.925	3.893	3.911	3.868
140	Disulfide, methyl 2-propenyl	0.625	0.214	0.224	0.231	0.216	0.245	0.253	0.254
141	Diallyl sulfide	0.546	0.241	0.239	0.235	0.217	0.214	0.218	0.207
142	Diallyl disulphide	0.529	3.174	3.269	3.225	3.204	3.266	3.342	3.264
143	Propanal, 3-(methylthio)	nd	0.201	0.194	0.192	0.185	0.188	0.191	0.186
144	Propane, 1-(ethynylsulfinyl)-	nd	1.028	1.025	1.034	1.021	1.01	1.018	1.093
145	Propanoic acid, 3-(acetylthio)-2-methyl-	nd	0.389	0.394	0.401	0.358	0.362	0.322	0.317
	Others								
146	Benzene, chloro-	nd	nd	nd	nd	nd	0.095	0.080	0.095
147	Cyclotrisiloxane, hexamethyl-	0.041	nd	nd	nd	nd	nd	nd	nd
148	Cyclotetrasiloxane, octamethyl-	0.378	nd	nd	nd	nd	nd	nd	nd
149	Cyclopentasiloxane, decamethyl-	0.096	nd	nd	nd	nd	nd	nd	nd
150	Cyclohexasiloxane, dodecamethyl-	0.161	0.088	0.123	nd	0.115	nd	nd	nd
151	Hydroxylamine, O-decyl-	0.026	nd	nd	nd	nd	nd	nd	nd
152	4-nitrophthalamide	0.369	nd	nd	nd	nd	nd	nd	nd
153	Silanediol, dimethyl-	1.611	nd	nd	nd	nd	nd	nd	nd
154	1-Heptadecanamine	0.041	nd	nd	nd	nd	nd	nd	nd
155	Phenol, 3-methyl-	0.026	nd	nd	nd	nd	nd	nd	nd
156	N-benzylidene-dimethylammonium chloride	nd	1.06	1.083	nd	nd	nd	nd	nd
157	Phenol, 2,4-bis(1,1-dimethylethyl)-	nd	nd	nd	nd	nd	0.439	0.209	0.165
158	Phenol, 2,6-bis(1,1-dimethylethyl)-4-met	nd	0.115	0.126	nd	nd	nd	nd	nd
159	Phenol, 4-methyl-	nd	0.111	0.048	nd	nd	nd	0.059	0.049
160	Phenol, 4-methoxy-	nd	nd	nd	0.055	nd	nd	nd	nd
161	Caffeine	nd	0.192	nd	0.173	nd	nd	nd	nd
162	Phenol, 2-methyl-5-(1-methylethyl)-	nd	nd	0.655	nd	nd	nd	nd	nd

163	Thymol	nd	nd	0.467	nd	0.224	nd	nd	nd
164	Phenol, 2-methoxy-	nd	nd	0.043	nd	nd	nd	nd	nd
165	1-Methoxy-2-propoxyethane	nd	nd	nd	nd	nd	nd	nd	0.160
166	1,3-Dioxolane, 4,5-dimethyl-2-pentadecyl	nd	nd	nd	nd	nd	nd	nd	0.082

A=Boiled specimens; B=Frozen storage for 0 month; C=Frozen storage for 1 month; D=Frozen storage for 2 month; E=Frozen storage for 3 month; F=Frozen storage for 4 month; G=Frozen storage for 5 month; H=Frozen storage for 6 month.
nd: Not Detected.

Table 2: *Relative content of volatile compounds of double-fried pork during cooking processing and frozen storage.*

	A (%) (101)	B (%) (91)	C (%) (89)	D (%) (83)	E (%) (84)	F (%) (81)	G (%) (84)	H (%) (89)
Aldehydes	60.738 (15)	51.287 ((13)	50.838 (12)	51.150 (12)	51.817 (12)	51.672 (12)	51.671 (12)	51.609 (12)
Alcohols	15.037 (26)	11.412 (15)	11.219 (16)	11.498 (15)	11.608 (16)	11.683 (16)	11.677 (15)	11.919 (15)
Hydrocarbons	6.859 (24)	4.030 (11)	3.362 (10)	2.776 (9)	2.439 (9)	1.894 (9)	2.332 (9)	2.358 (10)
Ketones	4.45 (9)	6.983 (6)	6.981 (5)	7.121 (6)	7.365 (6)	6.715 (3)	6.514 (6)	6.125 (6)
Esters	0.212 (6)	7.736 (12)	9.063 (10)	9.062 (10)	9.500 (10)	9.372 (10)	9.397 (10)	9.568 (12)
Acids	0.626 (7)	1.433 (4)	1.444 (4)	1.486 (4)	1.509 (4)	1.592 (4)	1.650 (4)	1.654 (4)
Heterocyclic compounds	0.357 (1)	6.873 (18)	6.898 (18)	7.146 (18)	7.331 (18)	7.229 (18)	7.297 (18)	7.149 (18)
Sulphur-Containing Compounds	7.337 (4)	9.397 (7)	9.636 (7)	9.504 (7)	9.126 (7)	9.178 (7)	9.255 (7)	9.189 (7)
Others	2.749 (9)	1.566 (5)	2.545 (7)	0.228 (2)	0.339 (2)	0.534 (2)	0.348 (3)	0.551 (5)

A=Boiled specimens; B=Frozen storage for 0 month; C=Frozen storage for 1 month; D=Frozen storage for 2 month; E=Frozen storage for 3 month; F=Frozen storage for 4 month; G=Frozen storage for 5 month; H=Frozen storage for 6 month.

thresholds will be greatly reduced with important effect to the flavor of food. In this study, 2-ethyl-5-methyl pyrazine, 2-ethyl-6-methyl pyrazine, 2,5-dimethyl-3-ethyl pyrazine and 2-methyl-3-ethyl pyrazine in double-fried pork had smell thresholds and significantly affected the flavor. Pyrrole compounds, similar in structure of furans as well as their formation mechanism, is mainly generate from the Maillard reaction. It is formed via 3-deoxidization ketone sugar reacted with amino compounds and ammonia or after dehydration condensation and cyclization reaction. 2-Acetyl pyrrole has grains aroma, but alkyl pyrrole and acyl pyrrole will produce bad flavor for double-fried pork.

Apart from heterocyclic compounds, esters also increased significantly. We found that post cooking, the relative contents of esters as well as their types significantly increased from 0.212% to 7.736%. However, we did not find ethyl caproate, ethyl methyl benzoic acid, benzoic acid, benzene acetic acid ethyl ester, 4-hydroxy butyric acid lactone, linalyl acetate, isobutyric acid orange flower ester in the boiled specimens, but found in the cooked specimens, indicating that these important flavor compounds were formed during the process of cooking, influencing the flavor of double-fried pork. Most of these compounds have a pleasant aroma of fruits and flowers; some have elegant and sweet, pure and fresh and powerful flavor. We performed PCA analysis for flavor volatile compounds of double-fried pork during frozen storage to determine the principal component of volatiles in them and to understand the influence of frozen storage processing on these compounds. We selected total 80 kinds of flavor compounds for double-fried pork with different frozen storage times; Table 3 shows the results of PCA. According to the characteristic value and variance contribution of the principal component (PC) we found that the cumulated variance contribution ratio of the first three PCs was 89.87%, and the average values of the first six PCs was more than 1. Moreover, according to the two principles of the determination of the numbers of PCs -cumulative variance contribution rates reaching up to 85% and the characteristic value higher than one

Table 3: *Eigenvalue, percentage of variance and cumulative of six principal components.*

Principal component	Eigenvalue	Extraction Sums of Squared Loadings	
		Variance (%)	Cumulative (%)
1	58.921	73.651	73.651
2	7.929	9.912	83.563
3	5.042	6.302	89.865
4	3.693	4.616	94.481
5	2.399	2.999	97.480
6	2.016	2.520	100.000

-we chose three PCs (PC1, PC2, and PC3) with contribution rates of 73.65%, 9.91% , and 6.30%, respectively. Table 4 presents the PC factor loading matrix; there are many flavor compounds - including benzaldehyde, geranial, dihydrogen celery alcohol, 1-octene-3-alcohol, linalool, *p*-xylene, 3-hydroxy-2-butanone, 2,3-symplectic diketone, methyl heptene ketone, ethyl caproate, ethyl lactate, ethyl methyl benzoic acid, benzoic acid, furfural, furfuryl alcohol, 2-amyl furan, 2,6-dimethyl pyrazine, 3-ethyl-2-2-acetyl methyl pyrazine, pyrrole, 2-acetyl furan, *N*-methyl pyrrole and allyl methyl sulfide - exerting very high loading in PC1. We found total of 63 volatile compounds positively related to PC1, including three aldehydes, twelve alcohols, seven hydrocarbons, ten esters, four ketones, three acids, seventeen heterocyclics, and seven sulfurs- the characteristic flavor compounds of double-fried pork. In addition, hexanal, nonanal, *trans*-2,4-sebacic olefin aldehyde and benzene ethanol had positive effect on PC2, whereas myrcene, caproic acid and 2-ethyl pyrazine had positive effect on PC3. In order to reflect the various compounds accounted for the proportion of each principal component clearly, we projected the points on three-dimensional load diagram (Figure 2) to the projection plane, thus achieving the two-dimensional load diagram (Figure 3). In Figure 3, PC1 represents the information of the majority of flavor compounds with few of them obtained from PC2. According to the synthesis score of double-fried pork.

***Table 4:** Loading matrix of the first three principal component factors.*

		Component		
		1	2	3
Pentanal	Q1	-0.338	0.297	0.493
Butanal, 3-methyl-	Q2	0.176	0.157	-0.225
Hexanal	Q3	-0.617	0.575	0.530
Heptanal	Q4	0.486	0.244	-0.253
Octanal	Q5	-0.366	-0.825	-0.001
Nonanal	Q6	-0.481	0.762	0.170
2-Octenal	Q7	0.881	-0.440	0.066
Benzaldehyde	Q8	0.886	0.252	-0.087
Benzeneacetaldehyde	Q9	0.511	-0.347	-0.722
Z-Citral	Q10	0.540	-0.481	-0.492
E-Citral	Q11	0.743	-0.362	-0.372
2,4-Decadienal, (E,E)	Q12	-0.033	0.753	-0.528
Ethanol	C1	-0.889	0.110	-0.373
Carveol, dihydro-, cis-	C2	0.875	-0.481	0.017
2-Propen-1-ol	C3	0.927	-0.121	-0.115
1-Butanol	C4	0.962	-0.126	-0.007
1-Penten-3-ol	C5	0.659	-0.706	0.052
1,8-Cineole	C6	0.921	-0.258	-0.013
1-Butanol, 3-methyl-	C7	0.452	-0.706	0.313
1-Pentanol	C8	0.954	-0.127	0.247
1-Hexanol	C9	0.962	0.233	0.103
1-Octen-3-ol	C10	0.987	-0.136	0.063
Linalool	C11	0.935	0.095	-0.193
1-Octanol	C12	0.990	-0.025	0.082
Terpinen-4-ol	C13	0.725	-0.518	0.238
2-Octen-1-ol-(E)	C14	0.957	-0.133	0.187
Phenylethyl Alcohol	C15	0.381	0.809	-0.152
Toluene	T1	-0.927	-0.139	0.070
Camphene	T2	0.921	-0.192	-0.234
Styrene	T3	0.946	0.220	-0.065
Benzene, ethyl-	T4	0.942	-0.125	-0.161
p-Xylene	T5	0.955	0.001	0.170
l-Phellandrene	T6	0.595	0.009	0.007
beta.-Phellandrene	T7	0.948	-0.054	0.081
beta.-Myrcene	T8	0.735	-0.133	0.608
l-Limonene	T9	0.943	-0.242	0.147
Acetone	K1	0.874	0.356	-0.272
2-Propanone, 1-hydroxy-	K2	0.917	0.040	0.068
2-Butanone, 3-hydroxy-	K3	0.960	0.220	-0.064
2,3-Octanedione	K4	0.973	0.017	-0.109
5-Hepten-2-one, 6-methyl-	K5	0.960	0.131	0.170
1-Butanol, 3-methyl-, acetate	E1	0.974	-0.099	0.107
Hexanoic acid, ethyl ester	E2	0.990	-0.068	-0.089
Propanoic acid, 2-hydroxy-,ethyl ester	E3	0.973	-0.041	0.197
Linalyl acetate	E4	0.791	-0.201	0.518
Propanoic acid, 2-methyl-, 3,7-dimethyl-	E5	0.943	-0.113	0.103
Benzoic acid, methyl ester	E6	0.985	-0.026	-0.039
2(3H)-Furanone, dihydro-	E7	0.955	0.134	0.089
Benzoic acid, ethyl ester	E8	0.971	0.138	-0.055
Benzeneacetic acid, ethyl ester	E9	0.958	0.238	-0.113
Butanedioic acid, diethyl ester	E10	0.955	-0.119	0.090
Acetic	A1	0.918	-0.243	-0.032
Butanoic	A2	0.937	-0.183	-0.106
Hexanoic	A3	0.408	0.176	0.822
Hexadecanoic acid	A4	0.897	0.336	0.287
Furan, 2-ethyl-	Z1	0.754	0.422	-0.334
2-Furancarboxaldehyde	Z2	0.974	0.149	-0.147
2-Furanmethanol	Z3	0.892	-0.102	0.202
2-Furanmethanol, 5-methyl-	Z4	0.734	0.439	-0.214
2-Furancarboxaldehyde, 5-methyl-	Z5	0.895	0.319	-0.006
Furan, 2-pentyl-	Z6	0.958	0.227	-0.025
Pyrazine, methyl-	Z7	0.940	-0.244	-0.120
Pyrazine, ethyl-	Z8	0.464	0.517	0.612
2,5-dimethyl pyrazine	Z9	0.914	0.262	0.032
Pyrazine, 2,6-dimethyl-	Z10	0.962	0.202	-0.047
Pyrazine, 2-ethyl-3-methyl-	Z11	0.863	0.465	-0.049
Pyrazine, 2-ethyl-5-methyl-	Z12	0.982	-0.110	-0.057
Pyrazine, 2-ethyl-6-methyl-	Z13	0.924	0.245	-0.013
Pyrazine, 3-ethyl-2,5-dimethyl-	Z14	0.919	0.218	0.047
Pyrazine, trimethyl-	Z15	0.908	0.387	-0.055
Ethanone, 1-(1H-pyrrol-2-yl)-	Z16	0.952	0.012	0.277
Ethanone, 1-(2-furanyl)-	Z17	0.937	0.251	-0.162
1H-Pyrrole, 1-methyl-	Z18	0.977	0.130	-0.049
2-Propen-1-thiol	S1	0.873	-0.137	-0.376
Disulfide, methyl 2-propenyl	S2	0.957	-0.249	0.130
Diallyl sulfide	S3	0.975	-0.008	-0.132
Diallyl disulphide	S4	0.973	0.162	-0.041
Propanal, 3-(methylthio)	S5	0.993	0.040	0.064
Propane, 1-(ethynylsulfinyl)-	S6	0.945	0.090	-0.041
Propanoic acid, 3-(acetylthio)-2-methyl-	S7	0.987	-0.029	0.006

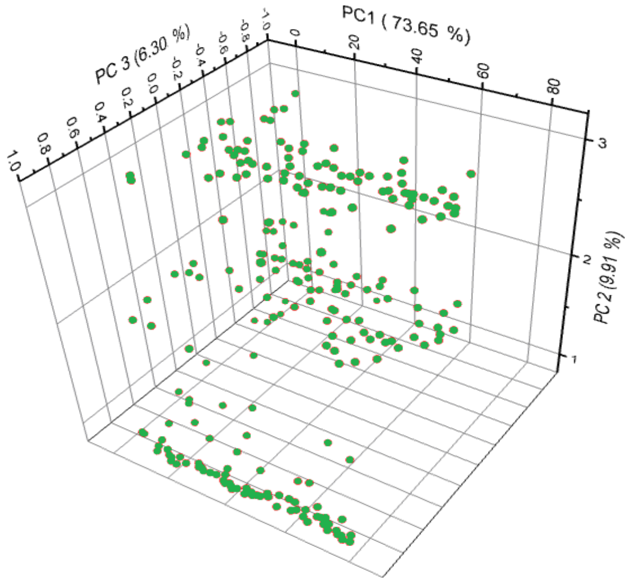

***Figure 2:** The dimensional loading diagram for the three principal components (PC1, PC2 and PC3) factors.*

The initial period of double-fried pork, before frozen storage, achieved the highest score. The score for double-fried pork decreased with increasing frozen storage times with the lowest score obtained for the double-fried pork sample stored for six months. Figure 3 illustrates the score plot of PC factor (PC1 and PC2), clearly distinguishing the flavor volatile compounds with different frozen storage times. As evident from Figure 4, the samples with frozen time of zero or one month, two months, three or four months and five or six months fall in the fourth quadrant, in the first quadrant, in the second quadrant and in the third quadrant, respectively. The main volatiles that affected the double-fried pork sample frozen for one month were 1-pentene-3-alcohol, isoamyl alcohol, caprylic aldehyde; the main volatiles that affected the double-fried pork sample frozen

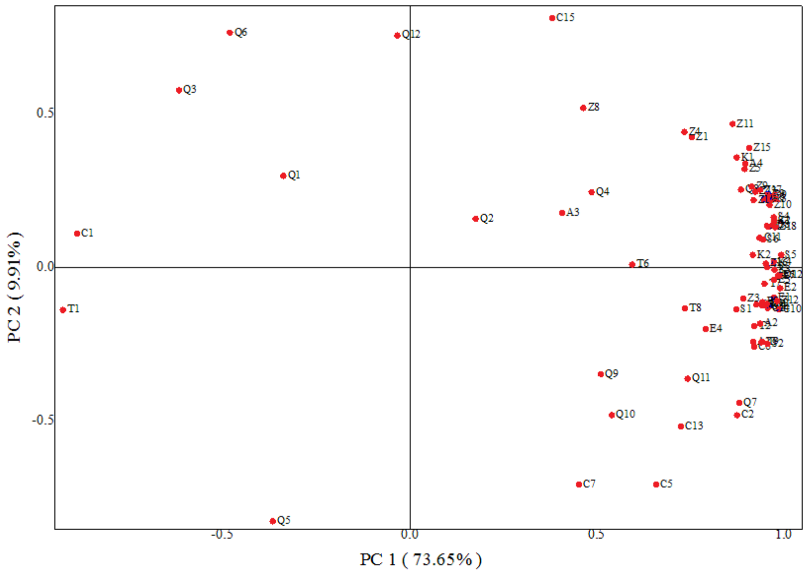

Figure 3: *The dimensional loading diagram for the two principal components (PC1 and PC2) factors.*

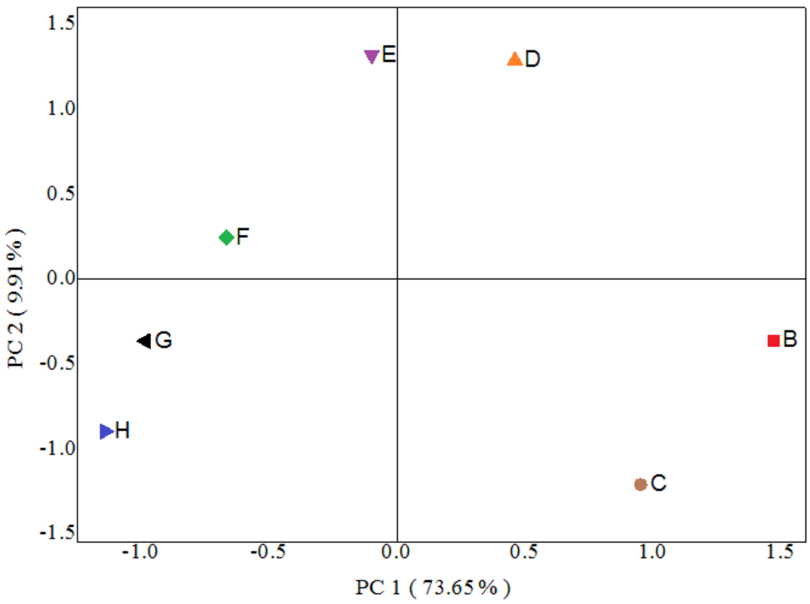

Figure 4: *Score plot of principal component factor. B=frozen storage for 0 month; C=frozen storage for 1 month; D=frozen storage for 2 month; E=frozen storage for 3 month; F=frozen storage for 4 month; G=frozen storage for 5 month; H=frozen storage for 6 month.*

for two or three months were nonanal, *trans*-2,4-sebacic olefin aldehyde, benzene ethanol; and the main volatiles that affected the double-fried pork sample frozen for five or six months were hexanal and ethanol.

Conclusions

We identified a total of 91 types flavor volatile compounds in double-fried pork - aldehydes (51.3%), alcohols (11.4%), sulfurs (9.4%), esters (7.7%), ketones (7.0%), heterocyclic compounds (6.9%), hydrocarbons (4.0%), and acids (1.4%). This study showed that heterocyclic compounds, esters, and aldehydes had a significant contribution to the overall flavor of double-fried pork, while alcohols, ketones and hydrocarbons had little effect. Post cooking, the most significant changes occurred in heterocyclic and esters contents. We found that the relative contents of heterocyclic compounds and esters increased from 0.36% to 6.87% and 0.21% to 7.74%, respectively and their types also increased from 1 to18 and 6 to 12, respectively. While the flavor profile of boiled specimens was dominated by grass aroma, fat aroma and fruit aroma, the aroma of cooked specimens was nutty, butyric, fruit and burnt. PCA analysis of the double-fried pork samples with different frozen storage times showed that PC1 represented the information for the most volatile flavors (73.7%), while the PC2 and PC3 represented little information for the volatiles. PC1 represented the characteristics of double-fried pork flavor; the synthesis score gradually decreased with increasing frozen storage time. The frozen storage time affected flavor compounds of the double-fried pork samples. During a frozen storage time of one month, the following flavor compounds were affected: 1-pentene-3-alcohol, isoamyl alcohol and caprylic aldehyde. Increasing the frozen storage time to two to three months affected mainly nonyl aldehyde, *trans*-2,4-sebacic olefin aldehyde and benzene ethanol. Furthermore, a frozen storage time of six months affected hexanal and ethanol.

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