

Optimization of Volatile Component Analysis Technique for Salt-Baked Chicken

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Abstract:

A Method for volatile components analysis of salt-baked chicken was established by using the headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) technology. The optimum process is determined by analysis of single factor. Specifically, the SPME fiber (carboxen/polydimethylsiloxane, CAR/PDMS, 75 μm) was used in the extraction of the volatile compounds in salt-baked chicken. Meantime, equilibrium time and extraction temperature were 10 min and 60 $^{\circ}\text{C}$, respectively. For the rest, the extractions were carried out for 30 min and desorption of the samples for 5 min. After preliminary analysis, 65 volatile components were detected in the salt-baked chicken. The extraction parameters were reasonable, feasible and practical, which was conducive to further qualitative and quantitative analysis of aroma substances in salt-baked chicken.

Key words: salt-baked chicken; volatile component; headspace solid-phase microextraction; optimization

1. Introduction

Salt-baked chicken is a Hakka dish with Cantonese characteristics in China. It is quite palatable and tender meat with high fat content [1]. As for the recipe of salt-baked chicken, kosher salt was used as the heat transfer medium to bake the chicken which had marinated with seasoning [2, 3]. Nowadays, the sales volume of salt-baked chicken was among the top of deli meat, which was made widely in our country. High baking temperatures and long cooking time would lead to a great thermal degradation/oxidation of lipids in salt-baked chicken, generating high content of lipid-derived volatile substances [4]. The flavour of the salt-baked chicken was an important indicator of its sensory quality. Therefore, it was of great significance to clarify the volatile components of salt-baked chicken, in order to further explore its flavor formation mechanism and improve its preparation technology. However, there were little research on the volatile components and flavour of salt-baked chicken at home and abroad.

Nowadays, solid-phase microextraction (SPME) has been widely used in odor research of deli meat products. SPME had the advantages of high efficiency, green and simple operation. A series of technological processes such as extraction, enrichment and injection could be

completed on the SPME device alone, which greatly reduced the analysis time. Meantime, compared with static headspace, it enriched volatile organic compounds at a lower concentration [5, 6]. Zhang et al. used electronic nose, electronic tongue and the headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) technology to evaluate the volatile flavor components of sugar-smoked chicken drumsticks, and 75 volatile compounds were identified [7]. The volatile compounds in braised pork with brown sauce were also revealed by HS-SPME-GC-MS, and 109 volatile flavour compounds were detected [8]. These data intuitively demonstrated the variation of the type and concentration of the volatile compounds that affect the flavour of meat products, which had an important effect on the exploration of flavour components.

The volatile flavor components of salt-baked chicken were complex and needed to be analyzed by precise instruments such as gas chromatography and mass spectrometry. Therefore, the detection method of SPME extraction technology combined with precise instruments has been recognized by researchers [9]. Consequently, the HS-SPME-GC-MS technique was manipulated to establish an analytical method for volatile

components of salt-baked chicken in this research. The optimum sampling conditions were investigated, including the variety of SPME fiber, the extracting temperature, the equilibrium time, the extraction time and the desorption time. Subsequently, the volatile flavor components of salt-baked chicken were preliminarily analyzed by the optimized process. This research explored the optimum technology of qualitative and quantitative analysis of aroma substances in salt-baked chicken, so as to provide experimental and theoretical basis for improving the flavour and promoting industrial production of salt-baked chicken.

2 Materials and methods

2.1 Ethics statement

The housing and treatment of the animals were carried out following national and international laws as well as with institutional guidelines. Since all chicken used in this study were obtained from a restaurant (Meizhou, Guangdong). We were explicitly issued a formal waiver of ethics approval.

2.2 Materials and reagents

Salt-baked chicken (white feather broilers) were purchased from a restaurant in Meizhou, Guangdong. 2-Methyl-3-heptanone (AR) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). A standard mixture solution containing C7-C40 hydrocarbons (1000 mg/L, dissolved in n-Alkane) was purchased from o2si smart solutions (Charleston, SC, USA).

2.3 Instruments and equipment

The extraction of the volatile compounds was performed using SPME with a manual microinjection needle (5190-1483, Guosheng Experimental Instrument Factory, China) and was separated, identified and quantified in a gas chromatograph (7890A GC-System, Agilent Technologies, Santa Clara, CA, USA) equipping with a mass selective detector (5975C MSD, Agilent Technologies). The magnetic stirrer (85-2A) was supplied by Guosheng Experimental Instrument Factory.

2.4 SPME Extraction

SPME fiber, extraction temperature, equilibrium time, extraction time and desorption time had the potential to affect the volatile aroma components. Meantime, the optimal detection conditions of the volatile aroma components of salt-baked chicken were explored by HS-SPME-GC-MS [10]. Briefly, the sample (4.0 g) was added to a 20 mL headspace vial (CNW Technologies, Duesseldorf, Germany). Next 2-methyl-3-heptanone (1 μ L; 0.812 μ g/ μ L in ethanol) was added as the internal standard (IS). The mixture solution was homogenized in a magnetic thermostatic water bath and the volatile compounds were detected by GC-MS.

2.5 Statistical analysis

Data are presented as the least-squared means of the three replicates with the standard error of the mean (SE). Statistical analysis was performed using Tukey–Kramer multiple-comparison tests as indicated in the table and figure legend. The JMP13 software was used to analyze the statistical significance level ($p < 0.05$, SAS Institute Inc., USA).

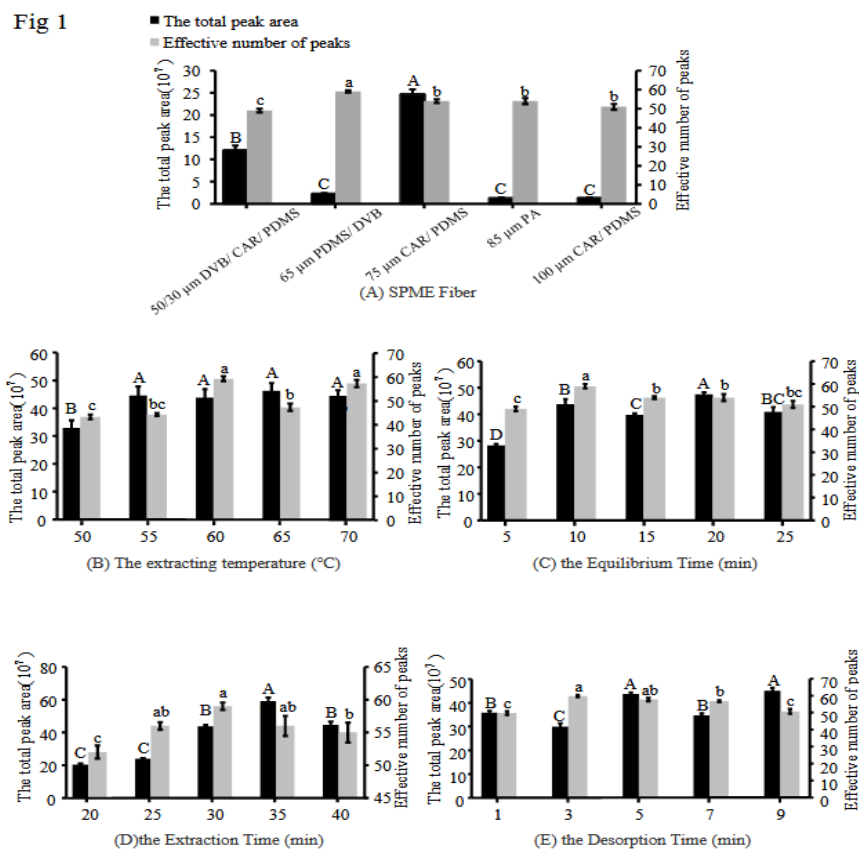


Figure 1: The effect of using different SPME conditions ((A) SPME fiber, (B) extracting temperature, (C) equilibrium time, (D) extraction time and (E) desorption time) on extraction efficiency. The total peak area and the effective number of peaks obtained by GC-MS were compared and analyzed. Values were shown as the mean \pm SE ($n = 3$). Different letters within the histogram of the same color indicates significant differences by Tukey–Kramer multiple comparison test ($p < 0.05$).

3. Results

3.1 The optimum conditions of SPME fibers

The SPME fiber is the core of the SPME extraction device. Different extraction coating materials have different adsorption capacities. The effects of 5 kinds of SPME fiber (50/30 μm thickness of divinylbenzene (DVB)/ carboxen (CAR)/ polydimethylsiloxane (PDMS), 65 μm thickness of PDMS/ DVB, 75 μm thickness of CAR/ PDMS, 85 μm thickness of polyacrylate (PA) or 100 μm thickness of CAR/ PDMS) on

chicken flavour compounds were explored. For the rest, the extractions were carried out for 10 min, after equilibration of the samples for 30 min, and desorption of the samples for 5 min at 40°C. As shown in Figure. 1(A), the fiber (CAR/PDMS, 75 μm thickness) showed greater adsorption capacity, which had the largest total peak area and more effective number of peaks. PDMS/ DVB (65 μm thickness) had the very small total peak area compared to the maximum value although it had the largest number of effective peaks. So, according to the total amount of volatile compounds, the fiber of 75 μm thickness of CAR/ PDMS was chosen in follow-up extraction experiments.

	RT (min)	Compound Name	CAS	Matching degree (%)	content ($\mu\text{g/g}$)	Threshold Value (mg/kg)
1	7.08	Hexanal	66-25-1	91	0.296322893	0.21
2	9.08	Meta-xylene	108-38-3	95	0.012761039	5.5
3	9.16	Ortho-xylene	95-47-6	89	0.005919554	3
4	9.86	2-Methyl-3-heptanone	13019-20-0	91	0.20298	-
5	10.49	Heptaldehyde	111-71-7	80	0.013436485	0.005
6	11.27	Cinene	005989-27-5	94	0.071761278	0.5
7	12.6	2-Pentylfuran	3777-69-3	93	0.043429049	0.0048
8	12.95	Styrene	100-42-5	97	0.022595713	0.022
9	13.57	2-Methylpyrazine	109-08-0	90	0.011440678	0.25
10	14.18	2-Octanone	111-13-7	94	0.004273606	0.05
11	14.25	Octyl aldehyde	124-13-0	91	0.094986742	0.0001
12	15.44	trans-2-Heptenal	57266-86-1	93	0.00421757	0.15
13	15.64	2, 3-Dimethylpyrazine	5910-89-4	86	0.00317469	0.1
14	15.84	2,3-Octanedione	585-25-1	80	0.174331578	-
15	16.81	Hexanol	111-27-3	90	0.066548497	0.2
16	17.53	3-Ethylpyridine	536-78-7	95	0.003454931	-
17	18.03	Nonyl aldehyde	124-19-6	94	0.121707269	0.0035
18	18.34	2, 3, 5-Trimethylpyrazine	14667-55-1	90	0.008431219	0.01
19	18.432	Tetradecyl	629-59-4	91	0.013979536	300
20	18.6	(Z)-3-Ethyl-2-methyl-1, 3-hexadiene	61142-36-7	94	0.026453806	-
21	19.11	2-Octene aldehyde	20664-46-4	84	0.032362144	0.05
22	19.74	3-Ethyl-2, 5-dimethylpyrazine	13360-65-1	93	0.007560117	0.025
23	19.88	Acetic acid	64-19-7	64	0.0041039	900
24	20.17	Furfural	98-01-1	87	0.164034001	0.01
25	20.36	Heptanol	111-70-6	80	0.034629878	0.2
26	21.7	Coumarone	271-89-6	93	0.038124762	-
27	21.84	Decanal	112-31-2	87	0.01612584	0.005
28	22.1	Pentadecane	629-62-9	96	0.004148139	-
29	22.41	Benzaldehyde	100-52-7	96	0.094545994	0.3
30	23.43	trans-2-Nonenal	18829-56-6	95	0.009296848	0.000065
31	23.59	3-Butyl-cyclopentanone	57283-81-5	96	0.009776674	-
32	24.64	2-Methyl-2,3-dihydro-1-benzofuran	1746-11-8	91	0.00119869	-
33	24.73	α -Cedrene	469-61-4	98	0.001751652	-

34	25.11	Octanol	111-87-5	87	0.067109201	0.054
35	25.58	2-Butylpyridine	5058-19-5	87	0.005290704	-
36	25.96	2-Methylbenzofuran	4265-25-2	94	0.009149949	-
37	27.43	2-Acetylpyrazine	22047-25-2	96	0.01017287	0.1
38	28.33	Acetophenone	98-86-2	93	0.037623924	3
39	29.59	Nonyl alcohol	143-08-8	91	0.012277373	0.002
40	29.73	O-Methylacetophenone	577-16-2	94	0.004693842	-
41	31.62	β -Bisabolene	000495-61-4	84	0.009819877	0.05
42	33.18	α -curcumene	644-30-4	98	0.003313802	-
43	33.57	Methoxy-phenyl-oxime		91	0.016621356	-
44	34.31	(E,E)-2,4-Decadienal	25152-84-5	95	0.008580047	0.00003
45	34.78	Anethole	104-46-1	98	0.037386118	0.1
46	35.62	Caproic acid	142-62-1	90	0.008312433	80
47	35.7	Guaiacol	90-05-1	97	0.010156153	-
48	36.19	Benzyl alcohol	100-51-6	97	0.001609666	5.5
49	37.25	γ -Symplectic lactone	104-50-7	90	0.003109064	0.095
50	37.91	3-(4-Methylphenyl)-2-propenal	1504-75-2	87	0.001750304	-
51	38.46	P-Methyl guaiacol	93-51-6	95	0.001777907	0.01
52	38.71	Heptanoic acid	111-14-8	81	0.001117007	0.1
53	38.86	2-Acetylpyrrole	1072-83-9	83	0.00084633	100
54	39.15	Diphenyl	92-52-4	91	0.001400275	-
55	39.81	Phenol	108-95-2	93	0.011791037	5.5
56	40.1	anisaldehyde	123-11-5	96	0.012729052	0.01
57	40.34	γ -Nonyl lactone	104-61-0	93	0.001260664	0.065
58	41.77	m-Cresol	106-44-5	95	0.001289311	0.002
59	42.69	Cedrol	77-53-2	98	0.001623855	-
60	43.04	Ethyl cinnamate	4192-77-2	98	0.000381516	0.00006
61	44.05	2-Ethylphenol	90-00-6	90	0.000574448	0.03
62	44.23	Pelargonic acid	112-05-0	93	0.001463142	1.5
63	45.04	Cadalene	483-78-3	95	0.000750931	-
64	45.93	ar-Turmerone	532-65-0	95	0.000690779	-
65	47.08	4-Methyl-5-beta-hydroxyethyl thiazole	137-00-8	97	0.014946091	10.8

RT: Retention Time; Matching value meant the matching degree of the compound to its chromatographic peak and mass spectrum peak; Threshold value meant the lowest concentration of the flavour substance which would be felt by human body.

Table 1: Qualitative and quantitative analysis of volatile compounds in salt-baked chicken

3.2 The optimum conditions of the extracting temperature

Extraction temperature is a significant factor in affecting extraction speed and efficiency. The effects of different extraction temperatures on the detection of volatile flavour compounds in salt-baked chicken were evaluated. Samples were extracted at different extraction temperatures (50°C, 55°C, 60°C, 65°C, 70°C) under the conditions of SPME fiber with 75 μ m, CAR/PDMS, fixing equilibrium time at 10 min, extraction time at 30 min and desorption time at 5 min.

When the extraction temperature was between 50-60°C, the number of detected effective peaks showed an increasing trend with a rise in temperature and reached the maximum value at 60°C (Fig. 1(B)). At the extraction temperature of 60°C, it had the biggest effective peaks. Meantime, the total peak area increased gradually with the increasing temperature, and became almost stable after 55°C. Interestingly, as the temperature further increased, the number of effective peaks decreased and reached a lower value at 65°C. Thus, 60°C was the optimal extraction temperature.

3.3 The optimum conditions of the SPME equilibrium time

Equilibration time was affected by interactions between the solid, liquid and gas phases in the sample. This step would explore the effect of different equilibration time on the detection of volatile flavour compounds in salt-baked chicken. Samples were extracted with different equilibrium times (5 min, 10 min, 15 min, 20 min, 25 min) fixing extraction time and desorption time at 30 min and 5 min, respectively. When the equilibration time was 10 min, the number of effective peaks reached the maximum (Fig. 1(C)). After 10 min, the number of effective peaks gradually decreased. So, the equilibrium time of 10 min was chosen in follow-up extraction experiments.

3.4 The optimum conditions of the SPME extraction time

The extraction time of SPME fiber is a key factor affecting the extraction results as well. The extraction was repeated by varying the extraction times (20 min, 25 min, 30 min, 35 min, 40 min). The desorption time was set at 5 min. As the figure 1(D) showed, the total peak area increased significantly with the prolonging of time until 35 minutes. After that, the total peak area tended to decrease. In addition, the number of effective peaks increased with the extraction time extension, and the peak value was obtained at 30 minutes. Generally, 30 min is the optimal extraction time with the largest number of effective peaks.

3.5 The optimum conditions of the SPME desorption time

This step explored the effect of different desorption times of the SPME fiber in the GC injection port on the detection results. Using the parameters determined above (SPME fiber, extracting temperature, equilibrium time and extraction time), chicken samples were extracted at different desorption times from 1 to 9 min (1 min, 3 min, 5min, 7min, 9 min). With the prolongation of the desorption time, the number of effective peaks represented as a bell-shaped curve, and reached the maximum value at 3-5 minutes (Fig. 1(E)). Furthermore, the total peak area was significantly high when the desorption time was 5 min. So, the desorption time was selected as 5 min.

3.6 Analysis of volatile components in salt-baked chicken

According to the optimized HS-SPME extraction conditions, the volatile components in the salt-baked chicken samples were extracted and desorbed. The volatile components in the salt-baked chicken samples were separated and identified by GC-MS. The qualitative and quantitative analysis of volatile compounds were performed by comparing MS with the NIST08 library (National Institute of Standards and Technology, Gaithersburg) and by comparing the chromatographic peak area of volatile substances with the internal standard [11]. Detailed quantitative principles of GC-MS referred to the article of Jiang et al [12]. According to the optimized SPME extraction conditions, 65 volatile flavour compounds were found in salt-baked chicken (table1). These volatile flavour compounds included aldehydes (12), alcohols (7), alkanes (12), heterocycles (15), ketones (7), acids (4), phenols (5) and esters (3).

4. Discussion

In the optimizing of the SPME fibers, the phenomenon which PDMS/DVB (65 μm thickness) had the very small total peak area and the largest number of effective peaks was also reported by Yu et al [13]. In the volatile flavour detection experiment of traditional smoke-cured bacon, it was also found that CAR/PDMS (75 μm thickness) coated fiber extracted the higher total peak area of volatile compounds than 65 μm thickness of PDMS/DVB and the PDMS/DVB (65 μm thickness) was given up because of its total peak area [13].

Next, a possible explanation for the phenomenon of the lower value at extracting temperature of 65°C was that high temperature would accelerate solvent containing flavour substances evaporation and some flavour substances were decomposed [14]. Meantime, high temperature

promoted the enrichment and adsorption of target compounds by SPME fiber. However, extraction is an exothermic process. As the temperature increased, the distribution coefficient of volatile substances between the SPME fiber and the sample would decrease, resulting in a reduced number of effective peaks. At the same time, the adsorptive capacity of the analyte and the sensitivity were reduced. This result was similar to the conclusion of Liang et, al [15]. The extraction efficiency showed an obvious increase from 40-80°C. However, when the extraction temperature was further increased to 100°C, no significant increase was observed [15]. Another possible explanation was that some volatile compounds were denatured and cracked at high temperatures, affecting the accuracy of the number of effective peaks [16].

Furthermore, the changes of volatile species in the chicken took time to reach equilibrium during the process of headspace absorption. Equilibration time was affected by the time for the sample to reach equilibrium in three-phase (the solid, liquid and gas phases) during the process of headspace absorption. The extraction efficiency increased with increasing equilibration time. However, the target sample would redissolve in the solvent if took a long time to equilibrate, resulting in lower extraction efficiency [16]. Interestingly, under the equilibration time of 20 min, the total peak area reached a maximum value. The phenomenon probably because some volatile compounds were redissolved with further increase in the equilibration time. This possible explanation needs to be confirmed in the future.

Moreover, the number of effective peaks and the total peak area increased with the extraction time extension before reaching the extraction equilibrium state. Therefore, during the extraction time of 35 minutes, some compounds might be redissolved, while some analytes continued to be adsorbed, so that the total concentration still increased. Li, T et al measured the effect of different extraction times of SPME on the extraction of volatile organic compounds (toluene, ethylbenzene and o-xylene) and found that the peak area of each analyte increased significantly within the extraction time of 5-15 min. However, excessive extraction times no longer increased extraction efficiency and sometimes caused desorption after 15 min [17, 18]. Similarly, at this moment, the degree of separation had shown a downward trend, and influenced the quantification of the peak area method.

What's more, in the matter of the SPME desorption time, the volatile substances evaporated at 3-5 minutes, but the concentrations gradually grew, which they could not be absorbed in a short time [19]. Meantime, too long absorption time would affect the service life of the SPME fiber [20]. Therefore, a lack of desorption time could easily lead to incomplete desorption, affecting the peak area. Some volatile aroma components would be decomposed with the passage of time. Thereby, the service life of the SPME fiber and the separation effect were affected.

Among the flavoring substances, hexanal, 2-methyl -3-heptanone, 2, 3-octanedione, furfural, nonyl aldehyde, octyl aldehyde and benzaldehyde were the main volatile components with high content. Meantime, the volatile flavour compounds (65) which detected by the optimum process condition were obviously more than the volatile flavour compounds (36) of salt baked chickens in research of Wu et al [21]. The results of this study provided credible data basis for the accurate evaluation of aroma components of salt-baked chicken, and provided reference for formation improvement and industrial production of salt-baked chicken.

Conclusions

In this research, the extraction conditions of SPME for volatile components analysis of salt-baked chicken were optimized. After thorough consideration, the volatile compounds of salt-baked chicken were extracted by SPME fiber of 75 μm , CAR/PDMS, equilibrium time and extraction temperature were 10 min and 60 °C. Furthermore, extraction time and desorption time were 30 min and 5 min, respectively.

Then the HS-SPME-GC-MS method was established and further volatile components in salt-baked chicken were detected. The result reflected 65 volatile flavour compounds were identified (12 aldehydes, 7 alcohols, 12 alkanes, 15 heterocycles, 7 ketones, 4 acids, 5 phenols and 3 esters). Based on the analysis of salt-baked chicken by HS-SPME-GC-MS, the material data affecting the flavour were obtained, which provided a theoretical reference for the preparation and industrial production of salt-baked chicken products.

Acknowledgments

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

None of the authors has any financial or other interest that could inappropriately influence or bias the content of this manuscript.

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