Data Statistics in Volatile Analysis by Accurate HS-SPME-GC/MS Measurement and Calculation

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Abstract. The volatile flavor compound in oil is an important indicator of quality which is widely used in the industry to assess the commercial value of oil. The volatile compounds of moringa seed oil were investigated by HS-SPME-GC/MS. The results showed that 52 volatile compounds were identified in moringa seed oil, including 12 hydrocarbons (20.66%), 3 acids (23.99%), 8 alcohols (8.28%), 2 esters (2.61%), 8 aldehydes (14.58%), 2 ketones (0.82%), 7 phenols (6.25%) and 10 heterocyclics (8.74%). Due to the low sensory threshold and high relatively content of aldehydes, aldehydes is one of the mainly factors to affect the flavor of moringa seed oil.

Keywords: moringa seed oil, volatile compounds, headspace solid-phase microextraction, gas chromatography –mass spectrometry.

1. Introduction

Moringa, a tropical and subtropical tree which is widely cultivated in Asia, South America, and Africa. Almost every part of moringa (fruits, flowers, leaves, roots, etc) has been proved that it is rich in nutrients [1]. The moringa kernel is rich in lipids reached 35% -40%. Due to the high level of unsaturated fatty acids, the olive oil is considered as nutritional and functional oil. Compared to olive oil, the fatty acid ingredients of moringa seed oil (MSO) was highly consistent with olive oil in terms of oleic acid [2]. Several methods can be used for the oil extraction from moringa seeds, such as low temperature press, soxhlet extraction, ultrasound-assisted and supercritical CO\textsubscript{2} extraction [3, 4]. The flavor is an important factor affecting the commercial value and edible quality of vegetable oil, and it is believed to be closely connected with the volatile compounds in oil [5]. Besides the growing environment, the processing technology and storage conditions are also affecting the ingredients and content of volatile compounds in oil [6]. Therefore, the identification of volatile compounds in MSO is very important to evaluate the quality and commercial value of MSO, which had successfully attracted the interest of researchers.

In this study, the MSO was prepared by solvent extraction method, and the volatile compounds in MSO were analyzed and quantified using an Agilent gas chromatography-mass spectrometry (GC/MS) combined with a headspace solid-phase microextraction (HS-SPME). This study is expected to identify the composition of volatile compounds in MSO and provide a crucial directive for the processing, storage and commercial application of MSO.
2. Materials and methods

2.1. Chemicals and instruments
Moringa seeds were purchased from Yunnan Yuanjiang Jianqiang Biological Technology Co. Ltd. Petroleum ether (30-60) was purchased from Tian in Fuyu Fine Chemical Co. Ltd. All other reagents were of analytical reagent grade.

GC-MS: 5890-5973 MSD, purchased from Agilent Technologies Co. Ltd (Shanghai, China); SPME Fiber: 50/30 μm DVD/Carboxen/PDMS, purchased from Supelco. (Shanghai, China); HP-5 capillary GC column: 60 m × 0.25 mm × 0.25μm, purchased from Agilent Technologies Co. Ltd (Shanghai, China).

2.2. Extraction of MSO
The MSO was extracted by solvent extraction method. In brief, 50 g of moringa seed power and 1000 mL petroleum ether were added into a flask, and stirred (150 r/min) by a magnetic stirrer at room temperature (25 °C) for 12 h. Then, the extract mixture was separated by centrifugal at 8000 r/min for 10 min. The moringa seed power was extracted three times under the same conditions. Ultimately, the extract was mixed together and petroleum ether was removed by rotary evaporator at 50 °C to obtain the MSO.

2.3. Volatile compounds analysis
According to the methods of Shuai X X, et. al [7], the pre-process of this study was conducted by HS-SPME, the volatile compounds in MSO was analyzed by GC-MS. The identification work was depended on the matching degree with the MS database, and the quantification work was operated through peak area.

3. Results and discussion
Volatile compounds are commonly present in vegetable oils as an important component for the flavor of oil, which consist of alcohols, aldehydes, ketones, alkanes, esters, acids and so on [8]. Because the flavor of oil is a crucial factor that influences the edible quality and consumer choice, the identification of flavor has caused great interest to scientists [5]. Furthermore, the HS-SPME-GC/MS technology is widely and efficiently used into the identification of chemical composition, especially in the evaluation of volatile compounds in oil. The volatile flavor components of MSO were shown in Table 1 and 2.
Table 1. The volatile flavor components in moringa seed oil

| Types       | Number | Volatile Components                        | Relative content% |
|-------------|--------|--------------------------------------------|-------------------|
| Hydrocarbons|        |                                            |                   |
|             | 1      | Butanal, 3-methyl-                         | 1.07              |
|             | 2      | Octane                                     | 9.87              |
|             | 3      | Heptane, 2,4-dimethyl-                     | 1.88              |
|             | 4      | Octane, 4-methyl-                          | 0.54              |
|             | 5      | Heptane, 2,4,6-trimethyl-                  | 0.34              |
|             | 6      | Octane, 3,3-dimethyl-                      | 0.52              |
|             | 7      | Undecane                                   | 1.56              |
|             | 8      | Decane                                     | 0.38              |
|             | 9      | 3-Ethyl-3-methylheptane                    | 0.53              |
|             | 10     | Hexane, 3,3-dimethyl-                      | 0.18              |
|             | 11     | Dodecanal                                  | 1.77              |
|             | 12     | Hexane                                     | 2.02              |
| Acids       | 13     | Acetic acid                                | 19.90             |
|             | 14     | Butanoic acid, 2-methyl-                   | 0.73              |
|             | 15     | Hexanoic acid                              | 3.36              |
| Alcohols    |        |                                            |                   |
|             | 16     | 2,3-Butanediol                             | 2.62              |
|             | 17     | 2,3-Butanediol, [S-(R*,R*)]-               | 0.79              |
|             | 18     | 1-Octanol                                  | 0.85              |
|             | 19     | 1-Hexanol                                  | 0.16              |
|             | 20     | Benzyl Alcohol                             | 1.42              |
|             | 21     | 1-Octanol                                  | 0.42              |
|             | 22     | 1,6-Octadien-3-ol, 3,7-dimethyl-           | 1.17              |
|             | 23     | Phenylethyl Alcohol                        | 0.85              |
| Esters      | 24     | Ethyl Acetate                              | 0.75              |
|             | 25     | Butyrolactone                              | 1.86              |
| Aldehydes   | 26     | Pentanal                                   | 3.73              |
|             | 27     | Heptanal                                   | 0.52              |
|             | 28     | Benzaldehyde                               | 1.92              |
|             | 29     | 2-Furancarboxaldehyde, 5-methyl-           | 3.39              |
|             | 30     | Octanal                                    | 0.92              |
|             | 31     | Benzeneacetaldehyde                        | 1.59              |
|             | 32     | Nonanal                                    | 2.35              |
|             | 33     | 2-Heptenal, (Z)-                          | 0.16              |
| Ketones     | 34     | Cyclohexanone                              | 0.49              |
|             | 35     | 5-Hepten-2-one, 6-methyl-                  | 0.33              |
| Phenols     | 36     | Toluene                                    | 0.40              |
|             | 37     | p-Xylene                                   | 0.33              |
|             | 38     | Phenol                                     | 0.80              |
|             | 39     | Benzene, 1-methyl-2-(1-methylethyl)-       | 0.49              |
|             | 40     | Benzyl nitrile                             | 3.53              |
|             | 41     | Naphthalene                                | 0.19              |
|             | 42     | Benzene, 1-methoxy-4-(1-propenyl)-        | 0.51              |
| Heterocyclics| 43     | Pyrazine, methyl-                          | 1.92              |
|             | 44     | Pyrazine, 2,5-dimethyl-                    | 1.10              |
|             | 45     | Pyrazine, 2,6-dimethyl-                    | 0.56              |
|             | 46     | Pyrazine, ethyl-                           | 0.96              |
|             | 47     | Pyrazine, 2-ethyl-6-methyl-                | 0.42              |
|             | 48     | Pyrazine, 2-ethyl-3-methyl-                | 0.31              |
|             | 49     | Pyrazine, 2,6-diethyl-                     | 0.33              |
|             | 50     | 2-Pyridinamine, N-methyl-                  | 1.11              |
|             | 51     | Furfural                                   | 1.66              |
|             | 52     | 2(3H)-Furanone, 5-ethylidihydro-           | 0.37              |
Table 2. Types of volatile flavor components in moringa seed oil

| Serial Number | Types          | Relative content% | Content of total volatile Flavor component% |
|---------------|----------------|-------------------|-------------------------------------------|
| 1             | Hydrocarbons   | 20.66             | 24.04                                     |
| 2             | Acids          | 23.99             | 27.92                                     |
| 3             | Alcohols       | 8.28              | 9.64                                      |
| 4             | Esters         | 2.61              | 3.04                                      |
| 5             | Aldehydes      | 14.58             | 16.97                                     |
| 6             | Ketones        | 0.82              | 0.95                                      |
| 7             | Phenols        | 6.25              | 7.27                                      |
| 8             | Heterocyclics  | 8.74              | 10.17                                     |

Table 1 and 2 exhibited the composition and types of volatile components in MSO. The results showed that 52 kinds of volatile compounds in MSO were identified and quantified, including 12 hydrocarbons (20.66%), 3 acids (23.99%), 8 alcohols (8.28%), 2 esters (2.61%), 8 aldehydes (14.58%), 2 ketones (0.82%), 7 phenols (6.25%), 10 heterocyclics (8.74%). The main volatile compounds were acetic acid (19.90%), octane (9.87%), pentanal (3.73%), benzyl nitrile (3.53%), 2-furancarboxaldehyde (3.39%), hexanoic acid (3.36%), 2,3-butanediol (2.62%), nonanal (2.35%) and hexane (2.02%), which accounted for more than 50% of the total volatile compounds in MSO. Hydrocarbons, acids and aldehydes were the major components among the volatile compounds that identified in this study, which accounted for 68.93% of the total volatile flavor components in MSO. Additionally, the esters, ketones, phenols and heterocyclics were much less than hydrocarbons (24.04%), acids (27.92%) and aldehydes (16.97%) in MSO, with the content of 9.64%, 3.04%, 0.95%, 7.27%, 10.17%, respectively.

Beyond cooking as edible oil, the MSO also exhibits strong antiarthritic, anti-inflammatory and antioxidant biological activity [9]. Compared with the flaxseed oil [10], the content of alkanes were significantly higher than others, while the volatile components in MSO were acids, which were accounted for 27.92% of the total volatile components. Due to the low sensory threshold of aldehydes, these compounds had significant impact on the overall aroma of vegetable oil [11], which usually presented a pungent smell of fat, fruit and spices. In this study, pentanal, 2-furancarboxaldehyde and nonanal occupied the largest proportion of aldehydes, which accounted for 25.58%, 23.25%, 16.12% of the total relative content of aldehydes in MSO, respectively. Furthermore, alcohols, heterocyclics and esters also play a positive role in overall aroma of plant oil [6], [11]-[12]. But the content of esters volatile components in MSO was only 2.61%, which maybe limited its impact on the overall aroma of MSO.

According to the study of Daviädek, carbonyl cleavage was the main way to produce acids volatile compounds [13]. And Morales found that the content of acids volatile compounds in spoilage oils were high [14]. So acids have a negative effect on the aroma of oil. Generally, hydrocarbons have a high sensory threshold, so it can be considered that the hydrocarbons had no significant contribution to aroma. Most hydrocarbons are one of monoterpenoids compounds and usually produced by biosynthesis method. The thermal oxidation of unsaturated fatty acids via hydroperoxides is the main pathway to generate ketones. Therefore, the ingredients of volatile compounds in oils were tightly related to the storage environment and processing conditions. The study will provide reference for the processing of MSO.

4. Conclusion
The flavor of oil is a crucial factor that influences the edible quality and consumer choice, while the volatile compounds are important component for the flavor of oil. In this study, HS-SPME-GC/MS was successfully applied for the analysis of volatile compounds in moringa seed oil. There were 52 volatile compounds in MSO were identified and quantified, including 12 hydrocarbons (20.66%), 3
acids (23.99%), 8 alcohols (8.28%), 2 esters (2.61%), 8 aldehydes (14.58%), 2 ketones (0.82%), 7 phenols (6.25%), 10 heterocyclics (8.74%). The acids, hydrocarbons and aldehydes had higher values than other volatile compounds, which accounted for 59.23% of the total volatile compounds. The sensory threshold and content of volatile compounds are the important factors effecting its contribution for overall aroma. Aldehydes maybe one of the mainly factors to affect the flavor of moringa seed oil.

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