Effects of different drying conditions on volatile components of *Pericarpium Arecae*

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Abstract. In the present study, the different drying temperatures and time on volatile components of *Pericarpium Arecae* (PA) were quantitatively and qualitatively analysed by headspace solid phase micro extraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). The matching similarity retrieval libraries and retention index (RI) were used to characterize the unknown compounds; and the contents of each component were determined by internal standard method. Finally, the significance among groups was inspected by through ANOVA and Duncan’s multiple range tests. As the results showed, 23 main characteristic volatile compounds were separated and identified, including esters, organic acids, aldehydes, alcohols and alkaloids. It was found that drying at different temperatures had a great impact on esters, organic acids, aldehydes and alcohols. And there was a significant difference among the groups. Therefore, through the accurate qualitative and quantitative analysis of volatile components in PA dry products, it is helpful to evaluate and judge the whole process, and provide a theoretical basis for its further development and utilization.

1. Introduction

*Pericarpium Arecae* (PA), the pericarp of *Areca catechu* L., an evergreen tree of *Palmae* family. PA originally from Southeast Asia has now mainly been cultivated in Hainan and Taiwan provinces in China [1]. Betel nut chewing is the fourth most popular habit in the world after tobacco, alcohol and caffeine; it is common in many parts of Asia [2]. The main edible part of areca nut is PA, which accounts for more than two-thirds of the fruit weight. Modern pharmacological studies have shown that PA has various effects including antiparasitic, antibacterial, antifungal, antioxidant, anti-allergic, anti-inflammatory and analgesic; as well as effects on digestive, nervous and cardiovascular systems; and regulatory effects on blood glucose and lipids et al. based on its wide spectrum of biological and pharmacological activities[3-4]. Volatile compounds are the important one of the indicators of evaluating crop quality which is directly affected the sensorial quality of fresh and processed products [5].

Due to the complexity of plant volatile components, it is impossible to use standard substances to identify them one by one. Moreover, the performance of each instrument and the separation efficiency of each column are obviously different. This causes a lot of interference factors to the mass spectrometry. Furthermore, because of the similar structure of the isomers, the mass spectra are almost the same. Therefore, there is great uncertainty in determining the chemical structure of the corresponding components only by using mass spectrometry to retrieve high matching degree.
Retention index (RI) of the same compound is usually a constant in different instruments as long as the column used for separation has the same properties and the samples are analyzed in similar chromatographic conditions. Therefore, using mass spectrometry and RI matching can greatly improve the accuracy of identification. In the identification of volatile complex components, this method has been widely recognized and used in the world [6-7]. In order to objectively evaluate the effect of drying temperature and time on volatile components of PA, headspace-solid phase micro extraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) combined with RI was used in this study. The current results will provide useful information for its further development and utilization.

2. Materials and methods

2.1 Plant materials, reagents and apparatus

The materials collected from Hainan province were identified as PA. The reagents used in this study included: n-alkanes std. (C7~C30) was acquired from Supelco (USA). α-pinene, hexane and methanol were supplied from Sigma (Germany).

The apparatus used in the study included: GCMS-QP2010Plus instrument equipped with a quadrupole mass analyzer (Shimadzu, Japan), AOC-5000 instrument equipped with headspace, solid-phase micro extraction (SPME) and liquid three in one automatic injector (Shimadzu, Japan). The fibre, coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30μm, Supelco, USA) was used for extraction. A fused silica capillary Rtx-5ms (Restek, USA) column (30 m × 0.25 mm×0.25 μm) was used for the separation.

2.2 Preparation of sample solutions

The same batch of PA sample was divided into five parts. One part (50 g) was a fresh sample without any handling (A). The rest four parts (50 g each) were respectively dried to less than 10% moisture by 40 °C, 6 h (B), 60 °C, 4 h(C), 80 °C, 2 h (D) and 100 °C, 1 h (E).

2.3 Sample extraction

Prior to sampling, the fibre was preconditioned at 250°C in the GC injection for 0.5 h. PA samples above (about 2 g each) with α-pinene (internal standard, IS) were successively put in a sealed vial. For balance, the vial was put in the temperature-controlled agitator tray at 90 °C for 5 min with magnetic stirring (250 rpm). Subsequently, the SPME device was automatically inserted into the sealed vial through the septum and the fibre was exposed to the sample headspace mode for 15 min under the same state. After extraction, the SPME device was introduced in GC and maintained at 250°C for 5 min.

2.4 Gas chromatography-mass spectrometry (GC-MS) analysis

The isolation, identification, and semi-quantification of the volatile compounds were performed on a GC-MS instrument. The GC injection and MS interface temperatures were respectively maintained at 250°C and 280°C. Electron ionization (EI) was used as the ion source; and the electron impact energy was 70 eV as well as the ion source temperature was 230 °C. The following temperature program was used with a 1 min solvent delay. Initially, the temperature began at 70 °C (held for 2 min) and then increased to 130°C (held for 2 min) at a rate of 10 °C/min, and then gradually increased to 280 °C (held for 2 min) at a rate of 15 °C/min using splitless mode. The constant flow rate of the carrier gas (Helium) was 1 mL/min. The EI mass spectra were set to full scan from 35 to 550 atomic mass units (m/z).

2.5 Compounds identification

2.5.1 Qualitative analysis.

The volatile compounds were identified by using National Institute of Standards and Technology mass spectral library(NIST 14) and Wiley Registry of Mass Spectral Data,
9th Edition (Wiley 9) with mass spectrometry database retrieval. (2) RI values were calculated with the help of n-alkanes. The RI values obtained were compared with the RI values of corresponding substances in literatures for qualitative analysis.

2.5.2 Quantitative analysis. Samples of each group were tested three times in parallel, and then all chromatographic peaks were integrated according to the basic integration rules. And then the relative content of each effective volatile component was processed. The ANOVA and Duncan’s Multiple Range Test were tested at the level of $p = 0.05$ with the analysis software IBM SPSS Statistics 21. The results were expressed as relative content (mean ± standard error).

3. Results and discussion

3.1 Qualitative analysis of volatile components of PA after drying

The volatile components were identified by comparing their relative retention times, mass spectra similarity, retention index and comparison with standards. Fig. 1 showed the total ion chromatogram (TIC) of the volatile components of PA after drying by HS-SPME-GC-MS. These 23 chromatographic peaks digitally labeled in the Fig.1 were typical volatile components of PA, obtained by similarity matching retrieval of mass spectrometry library and RI calculation. And the corresponding to the compounds was listed in Table 1. These compounds were composed of 12 esters, 4 organic acids, 2 aldehydes, 2 alcohols, 2 alkaloids and 1 ketone.

3.2 Quantitative analysis of volatile components of PA after drying at different conditions

The relative contents of volatile components in PA dried at different temperatures and times were listed in Table 1. With the increase of drying temperature and time, the total content of volatile components decreased from 1128.46 (μg/g) down to 730.82-751.97 (μg/g). There was no significant difference among the volatile components obtained from group C, D and E, all of which had significant effects on the volatile components of PA.

3.2.1. Esters. Their relative contents were accounted for nearly half of the volatile components (51.55%) in PA. In the literature [8], aldehydes were the main components in immature PA, which was different from the study. The main reason for the difference may be the origin and maturity of the selected experimental materials. As recently been reported [9], organic acids and esters were the main components in the volatile oil of PA, which was the same as the study. However, there were a lot of differences in types and contents of volatile compounds because of the different extraction methods. The total relative content of esters in group C, D and E were significantly lower than group A and B. There was no significant difference among the three groups; but there was significant difference between group A and B. The content of most volatile esters in PA decreased with the increase of drying temperature.

Among them, some esters such as ethyl elaidate (No.21) and ethyl linolenate (No.22) could not be detected with increasing drying temperature. Ethyl linoleate (No.20) was decreased with increasing drying time. However, the relative content of methyl-1-methyl-1,2,5,6-tetrahydropyridine-3-carboxylate (No.5) was raised up with the increase of the drying temperature. The compound is a derivative of arecoline, which may be obtained by arecoline heated.

3.2.2 Organic acids. The content of total organic acids was decreased with the increase of drying temperature. It was reported [10] that, tetradecanoic acid, oleic acid, linoleic acid, exadecanoic acid and dodecanoic acid were the main organic acids in PA. There were some difference in organic acid species between the experiment and the literature, maybe because of the different extraction methods. Among the 4 organic acids detected in this experiment, the trends of hydrocinnamic acid (No.8) and dodecanoic acid (No.11) were similar as that of total organic acids, and the difference was significant among the groups. Otherwise, the relative content of tetradecanoic acid (No.13) rose with the
increasing temperature, but there was no significant difference after 60 °C among the groups. And at the same time, the relative content of hexadecanoic acid (No.17) would be lowering when heated, but there was no significant difference among the groups.

3.2.3 Aldehydes and alcohols. There were significant differences between the total and the single content in each group, which the contents of aldehydes and alcohols decreased with the increase of drying temperature.

3.2.4 Alkaloids. It is always considered as the main physiological active ingredient of PA. In the literature [11], temperature had a great influence on content of arecoline. The longer the drying time was, the more the arecoline decreased. The relative content of arecoline (No.6) measured in the experiment decreased with the increasing temperature, which was consistent with the literature above.

In addition to arecoline, homoarecoline can be detected in the volatile alkaloids, which has not been similarly reported in most of the literatures. The relative content of homoarecoline (No.7) was measured in the experiment increased with the increasing drying temperature. This may be due to homoarecoline (C9H15NO2) had one more methyl group compared with arecoline (C8H13NO2); with the increasing temperature, the methylation of arecoline occurred at high temperature, which needs to be verified by further experiments. As Kai-Yue L [12] reported, Maillard reaction could be inhibited the demethylation of arecoline at high temperature through solid state reaction model, while it was possible to promote the methylation of arecoline at high temperature.

The total relative content of volatile alkaloids was showed a downward trend with the longer drying time. There was no significant difference between group A and group E, but the two groups had significant difference with the other three groups.

3.2.5. Ketones. There were significant difference between group A and other dried groups. But there was no significant difference among the dried groups. Drying temperature and time had an effect on the volatile ketones, which the effect was not linear correlation.

4. Conclusion
HS-SPME, as an advanced online pretreatment technology, integrates sampling, extraction, enrichment and sampling, it could collect volatile components without solvent extraction, which accords with the current development direction of green environmental protection fast and rapid detection. Combined with GC-MS, the quality and technology of food processing can be monitored online in real time; and the active compounds and technology of drug processing can be tracked.

In this research, the volatile compounds in PA were studied at different drying conditions. The similarity of spectral library retrieval combined with RI qualitative analysis was used, which has small error and realize the accurate qualitative analysis of the unknown volatile compounds in PA. It can make up for the shortcomings of the conventional qualitative retrieval method of GC-MS spectral library. Therefore, the accuracy of analysis is improved and the qualitative results are made more objective and reliable.

In this study, 23 typical volatile compounds were identified, which were more accurate compared with previous literatures. These volatile compounds endowed PA with pharmacological properties. The relative effect of drying time on PA is much less than drying temperature. Therefore, in the subsequent in-depth research of various drying processed of PA, low temperature technology should be considered. At the same time, the accurate qualitative and relative quantitative analysis of volatile compounds in dried PA is conducive to the overall evaluation and judgment of the process, and provides a theoretical basis for its further development and utilization.
5. Appendices

Figure 1. GC-MS TIC of the volatile components in dried PA

Table 1. Chemical content identified in the volatile components of dried PA

| No. | Compounds | Retention Time (RT) | Retention Index (RI) | Similarity | CAS | Relative content (μg/g) |
|-----|-----------|---------------------|----------------------|------------|-----|------------------------|
|     |           | Lab. | Ref. | A | B | C | D | E |     |
| 5   | Methyl-1-methyl-1,2,5,6-tetrahydropridine-3-carboxylate | 8.609 | 1168 | 1170 | 92 | 86447-15-6 | (9.36±0.74)a | (30.80±1.81)c | (41.05±1.26)c | (82.96±1.15)b | (94.10±1.62)a |
| 9   | trans-Ethyl cinnamate | 13.813 | 1474 | 1476 | 89 | 4192-77-2 | (31.7±1.44a) | (17.01±0.14b) | (15.16±0.15c) | (13.49±0.22d) | (14.45±0.24ed) |
| 12  | Ethyl dodecylate | 15.196 | 1594 | 1597 | 94 | 106-33-2 | (37.34±0.98a) | (27.31±0.51b) | (18.24±0.65c) | (10.66±0.30d) | (3.09±0.49e) |
| 14  | Ethyl tetradecanoate | 17.042 | 1793 | 1793 | 94 | 124-06-1 | (37.66±0.83a) | (36.27±0.12b) | (25.65±0.98c) | (17.93±0.43d) | (13.13±0.77e) |
| 15  | Ethyl cis-9-pentadecenoate | 17.64 | 1867 | 1865 | 94 | 56219-09-1 | (19.33±0.59a) | (14.17±0.47b) | (11.22±0.72c) | (0.81±0.23d) | (5.76±0.33e) |
| 16  | Ethyl pentadecanoate | 17.842 | 1994 | 1994 | 94 | 41114-00-5 | (19.81±0.71a) | (9.95±0.38b) | (8.10±0.79c) | (7.10±0.14d) | (7.10±0.24e) |
| 18  | Ethyl 9-hexadecenoate | 18.46 | 1975 | 1975 | 95 | 54546-22-4 | (42.65±0.46a) | (33.66±0.39b) | (29.56±0.78c) | (16.30±0.26d) | (7.10±0.26e) |
| 19  | Ethyl hexadecanoate | 18.6 | 1994 | 1994 | 94 | 628-97-7 | (162.22±5.40a) | (151.18±3.12b) | (84.23±6.80c) | (26.18±4.05d) | (20.85±2.98e) |
| 20  | Ethyl linoleate | 19.7 | 2155 | 2155 | 92 | 544-35-4 | (54.59±5.88a) | (10.25±2.18c) | (31.05±5.88d) | (91.86±3.63b) | (108.66±8.85a) |
| 21  | Ethyl elaidate | 19.825 | 2174 | 2174 | 91 | 6114-18-7 | (98.65±7.53a) | (52.60±4.49b) | (11.63±1.69c) | ND | ND |
| 22  | Ethyl linolenate | 19.844 | 2177 | 2173 | 90 | 1191-41-9 | (57.12±0.59a) | (4.23±0.77b) | (3.03±0.18b) | (2.67±0.34b) | (2.42±0.15b) |
| 23  | Ethyl octadecanoate | 19.947 | 2192 | 2193 | 91 | 111-61-5 | (11.32±2.94a) | (2.50±0.62b) | (3.03±0.18b) | (2.67±0.34b) | (2.42±0.15b) |
|     | organic acids (4) | | | | | | | | | |
| 8   | Hydrocinnamic acid | 12.007 | 1349 | 1347 | 95 | 501-52-0 | (6.25±0.34a) | (5.92±0.08a) | (4.92±0.25b) | (4.43±0.25c) | (4.38±0.14c) |
| 11  | Dodecanoic acid | 14.866 | 1564 | 1566 | 95 | 143-07-7 | (20.59±0.67a) | (15.38±0.25b) | (12.90±0.77c) | (10.46±0.39d) | (8.65±0.59e) |
| 13  | Tetradecanoic acid | 16.755 | 1760 | 1763 | 95 | 544-63-8 | (5.97±0.3c) | (8.21±0.11b) | (9.32±0.32a) | (9.49±0.23a) | (9.44±0.07a) |
| 17  | Hexadecanoic acid | 18.376 | 1964 | 1964 | 94 | 57-10-3 | (12.08±0.50a) | (4.83±0.22b) | (4.55±0.44b) | (4.68±0.40c) | (4.74±0.08c) |
|     | aldehydes (2) | | | | | | | | | |
| 1   | Benzaldehyde | 5.189 | 964 | 964 | 95 | 100-52-7 | (9.32±0.19a) | (3.96±0.23b) | (0.85±0.08c) | (0.74±0.49d) | (0.43±0.06d) |
| 2   | Benzeneacetaldehyde | 6.544 | 1047 | 1047 | 93 | 60-12-8 | (14.29±0.89a) | (11.38±1.14b) | (8.87±0.35c) | (5.24±0.38d) | (2.77±0.55e) |
|     | alcohols (2) | | | | | | | | | |
| 3   | Guaiacol | 7.301 | 1234 | 1234 | 95 | 90-05-1 | (23.61±0.71a) | (15.33±1.36b) | (9.72±0.31c) | (5.98±0.42d) | (3.20±0.63e) |
| 4   | Benzeneethanol | 7.752 | 1119 | 1118 | 97 | 625-35-0 | (10.79±0.52a) | (4.74±0.47b) | (3.78±0.14c) | (2.13±0.26d) | (1.55±0.40e) |
|     | ketone (1) | | | | | | | | | |
| 5   | Guaicol | 8.909 | 1234 | 1236 | 94 | 63-75-2 | (428.65±2.27a) | (362.21±1.48b) | (352.73±2.36c) | (330.97±2.71d) | (314.49±2.50e) |
| 6   | Homourea | 11.278 | 1304 | 1307 | 93 | 28125-84-0 | (6.09±0.24a) | (14.11±0.26b) | (31.22±1.48c) | (81.21±1.11d) | (119.63±1.59e) |
| 7   | Arecoline | 9.863 | 1234 | 1236 | 94 | 63-75-2 | (434.75±2.50a) | (376.33±1.62b) | (383.96±2.71c) | (412.173±2.68d) | (434.12±2.89a) |
10 trans-β-Ionone 14.107 1496 1498 90 79-77-6 (6.73±0.33)a (5.23±0.22)b (5.08±0.13)b (5.12±0.06)b (4.91±0.03)b
Total content (23) (1128.46±24.08)a (844.82±7.42)b (730.82±7.05)c (749.87±1.16)c (751.97±9.22)c

Note: The value of RI Lab was the RI of each component calculated by the determination of C7-C30 series n-alkanes. And the value of RI Ref was the reference RI (Rtx-5ms) searched in the literature. The different letters after the same line indicated that the relative content had significant difference at the level p=0.05.

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