Research Article

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Fast discrimination of avocado oil for different extracted methods using headspace-gas chromatography-ion mobility spectroscopy with PCA based on volatile organic compounds

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Abstract: To establish a method for fast discrimination of avocado oil for different extracted methods, the headspace-gas chromatography-ion mobility spectroscopy (HS-GC-IMS) combined with principal component analysis (PCA) was used to analyze non-target volatile organic compounds (VOCs). The results showed that 40 VOCs were identified, and the VOCs of the extraction method had a significant difference and had been well distinguished in PCA. The species and content of avocado oil obtained by squeeze method were more than the aqueous methods and supercritical carbon dioxide extraction methods (SC CO2). In addition, the different avocado oil had their characteristic compounds: the 2-acetylthiazole and ethyl propionate were the unique compounds in the avocado oil obtained by SC CO2. A rapid method for the determination of avocado oil obtained by different extraction methods based on HS-GC-IMS had been established, and the method was fast and simple and had a good application prospect in the prediction of avocado oil processing methods.

Keywords: avocado oil, extraction method, headspace gas chromatography ion mobility mass spectrometry, principal component analysis

1 Introduction

Avocado (Persea americana Mill.) is mainly distributed in tropical and subtropical areas within north-south latitude of 30° or less. It is not only a fruit but also a kind of woody oil [1]. At present, the main varieties are “Gwen,” “Hass,” “Maluma,” “Choquette,” “Lula,” etc., and “Hass” is the most important variety, which accounts for 80% of the world’s total acreage, and Mexico has an annual output of 5.6 million tons, accounting for 34% of the world’s total production. Other countries such as Indonesia, Peru, Dominican Republic, and Colombia are also the main producing countries, which are accounting for 30% of the world’s total production [2]. China’s avocado is now mainly distributed in Guangdong province, Hainan province, and Guangxi province. Among them, Guangxi Province is the best trials in China, and the planting area exceeds 5,000 hectares [3].

Avocado is rich in nutrients and has high fatty acid content in the pulp. They are mainly composed of various
monounsaturated fatty acids and polyunsaturated fatty acids, such as oleic acid, palmitic acid, and linoleic acid [4]. Because of its excellent physiological and medicinal functions, avocado oil is widely used in the cosmetics and food industries. For example, avocado oil could be digested and absorbed efficiently by human body. In addition, it could prevent arteriosclerosis caused by cholesterol accumulation and cerebral mitochondrial dysfunction caused by diabetes [5], and it is also used for skin diseases, such as eczema [6,7]. However, there are certain differences in the oil content of avocados in different verities, origins, and maturities, and there are also certain differences in the avocado oil obtained by different extraction methods [8,9]. According to the investigation, there is still no effective method to identify the processing method of the commercially available avocado oil. Different processing methods have a greater impact on the quality of avocado oil, and the price also varies greatly. Therefore, it was necessary to establish a method to quickly discriminate avocado oil.

Ion mobility spectrometry (IMS) is an analytical instrument used for identifying chemical ionic species, which is based on the different migration speeds of different gas-phase ions in the gas phase. It is a fast, convenient, and low-cost analytical method. The gas molecules are converted into charged ions in the ionized region and then enter the drift tube for differential analysis according to the different migration speeds of the gas phase ions in the electric field. Ion mobility spectroscopy has been used in the rapid detection of sesame oil adulteration in the food industry [10], egg freshness detection [11], olive oil shelf life prediction [12], etc. The high sensitivity and selectivity of IMS make it an analytical method that is suitable. High-selection gas chromatography (GC) combined with high-sensitivity ion mobility spectroscopy can combine the advantages of the two analytical methods and qualitatively analyze the compound through two-dimensional retention time. It is a powerful analytical tool with low detection limit (down to ppb level), such as short analysis time (just a few minutes), easy to use, low maintenance cost, and low cost, and 2D spectrum can show the testing result more clearly than the traditional analysis methods [13]. Jinming et al. used GC-MS technology to separate and identify 26 volatile organic compounds (VOCs) from avocado oil [14]. So far, there is no comparative study on the VOCs of avocado obtained by different methods and no research on the application of GC-IMS technology to the separation and identification of VOCs in avocado oil.

In this study, the VOCs of avocado oil were characterized by headspace GC-IMS. The fingerprints of the VOCs of avocado oil were obtained by three different methods, such as the pressing method, the supercritical extraction method, and the aqueous method, and cluster analysis for the main components. In addition, the HS-GC-IMS with principal component analysis (PCA) was performed to establish a method for fast discrimination of avocado oil for different extracted methods.

2 Experimental section

2.1 Materials

Avocados were purchased from Changdachang Super Shopping Mall in Zhanjiang city, fresh and mildew-free. When the outer skin changes from dark green to dark brown, it was taken out and used immediately for oil extraction experiments and performed three independent extractions for each method.

2.2 Squeezing extracted

Squeezing extraction was performed according to the Santana et al. [15] with some modification of the parameters. The avocado was peeled and pitted to obtain avocado pulp, and then the pulp was cut into pudding with a thickness of 1 cm, a length of 1 cm, and a width of 1 cm. The avocado pulp pudding was dried in a vacuum freeze dryer (Millrock ST85B3, Millrock Technology, Kingston, NY) for 72 h, the drying temperature was −40°C, and the vacuum was 0.009 MPa. After drying, it was pulverized and squeezed by the sing screw expeller (OP101, Shenzhen Yimeikang E-Commerce Co., Ltd., China) with hot-pressed modes. Then, the avocado oils were collected and centrifuged at 10,000 rpm for 10 min in a low-temperature high-speed centrifuge (GR22gII, Hitachi, Japan) to remove other impurities. Then, it was stored in a refrigerator at 4°C for detection and analysis.

2.3 Supercritical carbon dioxide extracted

Supercritical carbon dioxide extraction was performed according to the Corzzini et al. [16] with some modification about the parameters. Briefly, the avocado was peeled and pitted to obtain avocado pulp, and then, the pulp was cut into pudding with a thickness of 1 cm, a length of 1 cm, and a width of 1 cm. The avocado pulp pudding was dried in a vacuum freeze dryer (Millrock ST85B3,
Millrock Technology, Kingston, NY) for 72 h, the drying temperature was −40°C, and the vacuum was 0.009 MPa. The dried avocado was coarsely powdered, passed through 40 meshes, and then extracted in a supercritical carbon dioxide extractor (HSFE-5 + 1, Jiangsu Gaoke Pharmaceutical Equipment Co., Ltd.). The extraction temperature and pressure of grade I were 45°C and 5 MPa, the extraction temperature and pressure of grade II were 55°C and 21 MPa, the separation temperature and pressure of grade I were 50°C and 6 MPa, and the separation temperature and pressure of grade II were 30°C and 6 MPa, respectively. Then, the avocado oils could stand for 1 h to discharge carbon dioxide in the oil and be collected and centrifuged at 10,000 rpm for 10 min in a low-temperature high-speed centrifuge (GR22gIII, Hitachi, Japan) to remove other impurities. Then, it was stored in a refrigerator at 4°C for detection and analysis.

2.4 Aqueous extracted

Aqueous extraction was performed according to the Werman et al. [4,17] with some modification of the process parameters and technical processes. Weigh 1000 g of avocado pulp after peeling and coring, the ratio of material to water is 1:2, then milled in a colloid miller (ZVF300-GSR5/P7R5T4MD, Shanghai Cheoke Machinery Co., Ltd., China) for 1 min, and add 1:2 water to the cleaning machine, mix the slurry with the cleaning liquid and stir evenly, the pH of the slurry was adjusted to 8.0 with a 1.00 mol/L sodium hydroxide solution. After the slurry was stirred for 1.5 h in 75°C water bath, the slurry was centrifuged at 10,000 rpm for 10 min (GR22gIII, Hitachi, Japan). The upper layer of oil (containing the emulsified layer) was taken and stored at 4°C for 24 h, and then the oil was centrifuged at 10,000 rpm for 10 min. The upper layer of the oil was collected and stored at 4°C for detection and analysis.

2.5 Headspace GC ion mobility spectroscopy analysis

Headspace gas chromatography ion mobility spectroscopy (IF1-00110, G.A.S. Gesellschaft für analytische Sensorsysteme mbH) was described by Delgado et al. [18] with some modification about the parameters. Briefly, 0.2 g sample was placed in a 20 mL headspace bottle and heated in an incubator at an oscillation rate of 500 rpm, 40°C or 80°C for 20 min. Then, the samples were injected into a quartz capillary column (FS-SE-54-CB-1, 15 × 0.53 mm, 0.5 µm) by nitrogen at a programmed flow as follows: 2 mL/min for 2 min, 10 mL/min for 8 min, 100 mL/min for 10 min, and 150 mL/min for 15 min, and the syringe temperature was 85°C and injection volume was 500 µL. The compound to be analyzed was broken up into a positive ion mode by a 3H ionization source in the ionization chamber, and each spectrum was scanned 12 times on average. Then, the positive ions generated were separated in the drift tube for a second time, the length of the drift tube was 98 mm, the drift tube was operated at a constant temperature of 45°C, and a voltage of 500 v/cm. The drift gas (nitrogen) was set to 150 mL/min. All analyses were repeated three times.

2.6 Statistical analysis

All experimental data were analyzed and plotted using the Laboratory Analytical Viewer, Gallery Plot plug-ins, Dynamic PCA plug-ins, and NIST database and IMS database provided with the equipment to analyze the avocado oil volatile compounds.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 HS-GC-IMS 3D topographic visualization spectra of avocado oil from different extraction methods

The 3D topographical visualization spectra of VOCs produced by different extraction methods of avocado oil are shown in Figure 1, where the X-axis represented the ion migration time used for identification, the Y-axis represented the retention time of the gas chromatograph, and the Z-axis represented the peak height used for quantification. The color indicated the concentration of the compound, white indicated the low concentration, and red indicated a high concentration [19]. Figure 1 shows that there were obvious differences in the VOCs produced by avocado oil obtained by different extraction methods. The squeezing extraction method had the largest number of peaks and the highest intensity.

The difference comparison model was used to compare the differences in avocado oil samples, as shown in Figure 2. The figure was according to the topographic plot of avocado oil obtained from squeeze extraction method as a reference, and the 3D topographical visualization
spectra of other samples was drawn. If the content and the compound concentration of VOCs were identical, the background would be white. If the compound concentration was higher than the reference value, it was indicated in red. If the compound concentration was lower than the reference value, it was indicated in blue. It could be seen that the retention time of VOCs in avocado oil obtained from different extraction methods was concentrated in the range of 100–400 s, and each sample had several high signal intensity peaks. This may be because the different extraction methods may lead to changes in fatty acids composition and the species and content of VOCs of avocado oil samples [20–22]. Avocado oil obtained from squeeze method had the most kinds of VOCs and the highest signal intensity than other methods, and compared with the other two methods, the aqueous extraction method had a fewer number and lower intensity of peaks.

The GC-IMS integration parameters of VOCs are shown in Table 1. Forty VOCs were detected, including 14 kinds of aldehydes, 13 kinds of alcohols, five kinds of ketones, three kinds of acids, three kinds of esters, and two kinds of heterocycles. Table 1 and Figure 3 show that the VOCs of avocado oil were mainly composed of

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Figure 1: 3D topographical visualization view of different extraction methods: (a) squeezing extracted; (b) supercritical carbon dioxide extracted; (c) aqueous extracted.

Figure 2: 3D-topographic (top view) of different extraction methods: (a) squeezing extracted; (b) supercritical carbon dioxide extracted; (c) aqueous extracted.
aldehydes and alcohols, and the squeezed method had the largest proportion of alcohol compounds, the supercritical extraction method had the largest proportion of ester compounds, and the aqueous method had the largest proportion of aldehyde compounds. Fernanda et al. [23] research showed that vacuum drying at 60°C had better quality than solvent extraction, which further proved that the extraction method had a greater impact on the quality of avocado oil. It could be preliminarily inferred that heat treatment promoted the increase in the content of alcohol compounds in avocado oil. The aqueous method had less damage to the oil flavor and retained the original aldehyde flavor compounds in the avocado oil to a greater extent. The supercritical carbon dioxide extraction method had the most esters compound content.

Table 1: GC-IMS integration parameters of VOCs

| Count | Compound     | CAS   | Formula | Mr   | Ri   | RTb (s) | DTc | Identification approach | Classification |
|-------|--------------|-------|---------|------|------|---------|-----|-------------------------|----------------|
| 1     | 1-Propanol   | C71238 | C₃H₇O  | 60.1 | 525.9| 112.915 | 1.1127 | RI, DT                  | Alcohols       |
| 2     | Butanal      | C123728 | C₃H₅O  | 72.1 | 576  | 132.207 | 1.2794 | RI, DT                  | Aldehydes      |
| 3     | 2-Butanone   | C78933  | C₄H₈O  | 72.1 | 588.8| 137.727 | 1.2449 | RI, DT                  | Ketones        |
| 4     | Ethyl acetate| C141786 | C₄H₈O₂ | 88.1 | 610.1| 147.524 | 1.3376 | RI, DT                  | Esters         |
| 5     | 2,3-Butanediol| C41038  | C₅H₁₀O₂| 86.1 | 627.8| 156.264 | 1.1666 | RI, DT                  | Ketones        |
| 6     | Propanoic acid| C79094  | C₃H₆O₂ | 74.1 | 672.8| 181.222 | 1.1051 | RI, DT                  | Acids          |
| 7     | Pentanal     | C110623 | C₅H₁₀O  | 86.1 | 687.5| 190.336 | 1.4251 | RI, DT                  | Aldehydes      |
| 8     | Ethyl propionate| C105373 | C₅H₁₀O₂| 102.1| 701.5| 199.471 | 1.4544 | RI, DT                  | Esters         |
| 9     | 2-Methyl butanol| C137326 | C₅H₁₂O | 88.1 | 703.3| 200.684 | 1.2338 | RI, DT                  | Alcohols       |
| 10    | 3-Hydroxy-2-butanol| C513860 | C₅H₁₀O₂| 88.1 | 706  | 202.494 | 1.3294 | RI, DT                  | Ketones        |
| 11    | 1-Pentanol M | C71410  | C₅H₁₀O  | 88.1 | 766.1| 248.277 | 1.2513 | RI, DT                  | Alcohols       |
| 12    | 1-Pentanol D | C71410  | C₅H₁₀O  | 88.1 | 763.8| 246.345 | 1.5114 | RI, DT                  | Alcohols       |
| 13    | 2-Hexanone   | C591786 | C₆H₁₂O | 100.2| 784.5| 264.432 | 1.4995 | RI, DT                  | Ketones        |
| 14    | n-Hexanal    | C66251  | C₆H₁₄O | 102.2| 852.4| 334.489 | 1.6069 | RI, DT                  | Aldehydes      |
| 15    | 2-Furfural   | C98011  | C₅H₈O   | 96.1 | 829.8| 309.24  | 1.3298 | RI, DT                  | Aldehydes      |
| 16    | 3-Methyl-1-pentanol| C589355 | C₆H₁₂O | 102.2| 854  | 336.422 | 1.5161 | RI, DT                  | Aldehydes      |
| 17    | E 2-Hexen-1-ol| C2305217| C₆H₁₄O | 100.2| 855.4| 338.055 | 1.18   | RI, DT                  | Alcohols       |
| 18    | 2-Hexen-1-ol | C2305217| C₆H₁₄O | 100.2| 854  | 336.422 | 1.5161 | RI, DT                  | Aldehydes      |
| 19    | 1-Hexanol    | C111273 | C₆H₁₂O | 102.2| 874.3| 361.204 | 1.6458 | RI, DT                  | Alcohols       |
| 20    | 2-Methylbutanoic acid| C116530 | C₆H₁₂O | 102.2| 876.9| 364.418 | 1.4714 | RI, DT                  | Ketones        |
| 21    | 2-Heptanone  | C110430 | C₇H₁₄O | 114.2| 939.5| 386.811 | 1.6263 | RI, DT                  | Ketones        |
| 22    | Methional    | C3268493| C₇H₁₄O | 104.2| 904.5| 401.544 | 1.4009 | RI, DT                  | Aldehydes      |
| 23    | Heptanol M   | C111717 | C₇H₁₄O | 114.2| 905  | 402.206 | 1.3371 | RI, DT                  | Aldehydes      |
| 24    | Heptanol D   | C111717 | C₇H₁₄O | 114.2| 905  | 402.729 | 1.6945 | RI, DT                  | Aldehydes      |
| 25    | Methyl hexanoate| C106707 | C₇H₁₄O | 130.2| 936.8| 401.467 | 1.2872 | RI, DT                  | Esters         |
| 26    | 3-Methyl valeric acid M| C105431 | C₇H₁₄O | 112.2| 944.9| 463.152 | 1.2686 | RI, DT                  | Acids          |
| 27    | 5-Methylfurfuryl alcohol| C3857258| C₈H₁₄O | 112.1| 967.7| 502.194 | 1.5676 | RI, DT                  | Aldehydes      |
| 28    | E 2-Heptenal | C18829555| C₈H₁₄O | 112.2| 968.1| 502.843 | 1.6661 | RI, DT                  | Aldehydes      |
| 29    | Benzaldehyde | C100527 | C₇H₆O  | 106.1| 968.9| 504.255 | 1.4691 | RI, DT                  | Aldehydes      |
| 30    | 1-Octene-3-ol| C3391864 | C₈H₁₆O | 128.2| 1006.2| 606.668 | 1.4106 | RI, DT                  | Aldehydes      |
| 31    | 2-Pentylfuran| C377693  | C₈H₁₂O | 138.2| 1006.2| 575.812 | 1.2517 | RI, DT                  | Heterocyclics  |
| 32    | Octanal      | C24295032| C₉H₁₈O | 154.3| 1024.5| 614.878 | 1.2941 | RI, DT                  | Aldehydes      |
| 33    | 1,8-Cineole M| C470826  | C₉H₁₈O | 154.3| 1024.5| 614.878 | 1.2941 | RI, DT                  | Aldehydes      |
| 34    | 2-Acetylthiazole| C24295032| C₉H₁₈O | 154.3| 1024.5| 614.878 | 1.2941 | RI, DT                  | Aldehydes      |

* The retention index calculated using n-ketones C₄–C₉ as external standard on FS-SE-54-CB-1 column. b The retention time in the capillary GC column. c The drift time in the drift tube.
3.2 Differences in VOCs of avocado oil from different extraction methods

To quickly identify the avocado oil samples by equipment analysis, fingerprint information technology was used to qualitatively analyze all information contained in the different samples. These compounds were confirmed by comparing the IMS drift time and retention index with those of the reported and authentic reference compounds. Because of the difference in the concentration of VOCs, it could be observed that these compounds could produce multiple signals or spots, and 40 typical target compounds were identified (Figure 4 and Table 1). They were represented by Arabic numerals in Figure 4. Jinming [14] investigated the VOCs of avocado oil and its enzymatic hydrolysates using HS-SPME-GC/MS and 26 VOCs were identified. Only heptaldehyde, furfural, nonanal, and propionic acid were detected together, and the other components had a large difference. The reason for the difference between the two was that the samples used by Jinming were directly bought in the market and might be prepared. However, all the samples used in this study were obtained through direct extraction and did not undergo refining and other processes. Pedreschi [24] showed that avocado maturity had a large effect on the fatty acid composition of avocado oil, and the reason for the difference might also be derived from the maturity of the extracted oil raw materials. Giuffrè et al. [25] showed that heating temperature and time had significant effects on the content of VOCs in olive oil.

Fingerprint spectra were often used to visually observe the differences between different samples. The difference in avocado oil obtained by three different extraction methods could be observed through Figure 5. Each row represented all the signals peaks of one sample, and each column represented the signal peaks of the same VOCs in different samples. The color is represented the signal intensity of the substance; the darker the color was, the greater the intensity was. As could be seen from Figure 5, in the green dotted box, the signal intensity of butanal, 2-methyl butanol, 2-phenylacetaldehyde, 1,8-cineole, methyl hexanoate, benzyl alcohol, 3-methyl-1-pentanol, E 2-hexen-1-ol, 2-methylbutanoic acid, E,E 2,4-heptadienal, 2-furfural, 2-hexanone, 2-butanone, 3-hydroxy-2-butanone, and 2,3-butanediene were the highest in avocado oil obtained by squeeze method. Although the highest signal could be indeed observed in the squeezing extract, they were also other VOCs observed in the other extraction methods, such as 3-hydroxy-2-butanone or 2-butanone in Figure 5. In the light blue-dotted box, the signal intensities of 2-acetylthiazole, ethyl propionate, ethyl acetate, 2-heptanone, and 1-hexanol were the highest in avocado oil obtained by supercritical carbon dioxide extraction method. In the purple box, the signal intensities of 2-heptenal, 2-pentylfuran, benzaldehyde, 1-pentanol, 5-methylfurfuryl alcohol, 2-octenal, 3-methyl valeric acid, heptanal, and pentanal were the stronger in avocado oil obtained by the aqueous method. Gerhardt et al. [26] had effectively distinguished
the types of olive oil with the difference in VOCs produced from different samples. Therefore, it could be used to distinguish which extraction method was used to produce avocado oil by HS-GC-IMS fingerprints.
3.3 Cluster analysis of VOCs in avocado oil obtained by different extraction methods

PCA is a statistical analysis method. By orthogonal transforming, many interrelated original variables are converted into a few orthogonal principal component variables, and then the difference between the samples could be distinguished based on the contribution rate of principal component variables [27]. PCA was found using the signal intensities of different compounds and highlighted the differences in compounds.

This method had been successfully applied in vegetable processing [28], fruit identification and classification analysis [29], fruit and vegetable storage [30], and other aspects. The PCA of VOCs in the avocado oil obtained from different extraction methods is presented in Figure 6. It could be seen from Figure 6 that the first and second main component variance contribution rates were 52 and 44%, respectively, and the cumulative contribution rate was greater than 90%, and there were obvious differences between the three different samples.

To prove whether the data and sample from three replicates in parallel were valid, the similarity analysis was performed on the sampled data. As shown in Table 2, the analysis results showed that the sample obtained in this experimental sampling had a high degree of similarity and the data sampling was reasonable.

The PCA results showed that the avocado oil samples obtained from different extraction methods would be well distinguished in Figure 6. When the value of PC1 was greater than 2,000, it could be judged as avocado oil obtained by squeeze method. When the value of PC1 was less than −2,000, it could be judged as avocado oil obtained by supercritical carbon dioxide extraction method. When the value of PC1 was greater than −2,000 and less than 1,000, or the value of PC2 was greater than 2,000, it could be judged as avocado oil obtained by aqueous method.

Figure 6 shows that the avocado oil obtained by different extraction methods could be clearly distinguished. Moreover, the VOCs fingerprints of samples from different extraction methods were successfully set up through HS-GC-IMS. The various compound data obtained by GC-IMS analysis contained a lot of useful information, and the useful information obtained after these screenings could be used as a useful tool to distinguish avocado oil samples obtained from different extraction methods.

![Figure 6: PCA plot of VOCs in avocado oil obtained with the different extraction methods: (a) squeezing extracted; (b) supercritical carbon dioxide extracted; (c) aqueous extracted.](image-url)
4 Conclusion

In this study, 40 VOCs were identified by GC-IMS in avocado oil obtained from different extraction methods. The VOCs produced from avocado oil obtained by different extraction methods had a very significant difference, and each sample had unique compounds. In addition, the PCA and similarity analysis results showed that the avocado oil samples were independent from each other and each sample had unique compounds. In addition, the same time, this method could be used for the identification and classification of avocado oil samples.

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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