

Chemical Classification on Animals and Plants for Exploitation Based on Analysis of Fatty-Acid Compositions of their oils

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Abstract. To study chemical classification of animals and plants for exploitation, fatty-acid compositions of 23 plant oils and 16 animal oils extracted by supercritical CO₂ fluid extraction were determined by gas chromatography-mass spectrometry method(GC-MS) and these compositions were analyzed by Hierarchical cluster to study the chemical classification to these plants and animals. The result showed that the Hierarchical cluster method can be used to study chemical classification of animals and plants for exploitation and special fatty acid source can be classified by this method. the different characteristics of fatty-acid compositions between animals and plant are found by this method.

Keywords: chemical classification, fatty-acid compositions, oil, GC-MS, supercritical CO₂ fluid extraction

1. Introduction

Lipids can be found both in plant seeds and in animal fat and the main components are belong to different fatty acids. Unusual fatty acids are often present in significant percentages in the seed fat, but are almost totally absent in leaves and other parts of the plant. These interesting fatty acids and their sporadic occurrence in seed lipids is genetically determined, and they are highly significant indicators of phylogenetic relationships^[1]. The fatty acid composition of the two related and often synonymized *Kindbergia* species strongly suggested that they are chemically distinguishable and, thus, could be treated as separate entities^[2].The chemical components can be used to classify species such as farinose wax for Pteridaceae^[3], phenyl pyrone for 380 species of *Aloe*^[4], diterpenes for *Dictyota*^[5], n-alkyl lipid distributions and principal component analyses for *Quercus*, *Platanus*, *Magnolia*, *Pseudofagus*, *Fagus*, *Cocculus*, *Taxodium*, *Metasequoia* and *Sequoia*^[6], secondary metabolite profiling in chemotaxonomy of filamentous fungi^[7], leaf flavonoids, essential oils and furanocoumarins for tribe Psoraleae^[8], essential oils for Clerodendrum^[9], and other chemical components including phenolic chemotaxonomy^[10], essential oils^[11], alkaloids^[12], flavonoids^[13], glucosides^[14], and fatty acids were also reported for chemotaxonomy^[15-16], most of them used to plants not use animals. To exploit the application fields to fatty acids as a chemical components to chemotaxonomic classification, some fatty acids from animals and plants were determined for classifying their class recording to different fatty acids to evaluate their further exploitation.

2. Materials and Methods

2.1. Materials and Reagents

(1) Materials

The seeds were selected from market and animal tissue was obtained from fresh animal.

The dried seeds or parts of plants were ground in a blender to produce a powder and stored in dark at 4 °C.

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Fresh tissue homogenate of animals was mixed with diatomaceous earth to be extracted by supercritical CO₂ and stored in dark at 4 °C.

(2) Reagents

Analytical grade methanol, ether, benzene, n-hexane, petroleum ether, sodium hydroxide were purchased from Tianjin Chemical Reagent No 1 Factory, China. Carbon dioxide (99.99% purity) was bought from Jilin Baicheng Gas Co., Ltd. (Jilin, China).

2.2. Methods

(1) Supercritical CO₂ fluid extraction of oils

Instrument: A HA120-50-01 system (Nantong Hua-an Supercritical Extraction Co., Ltd., China) was used for supercritical CO₂ fluid extraction.

Condition: The extraction tube was a 1L stainless steel tube. Supercritical fluid extractions were conducted at pressures of 30 MPa and temperatures of 45 °C, extracted for 3-5h, in dynamic mode. The supercritical CO₂ flow rate was about 30 mL/min.

Plant sample preparation: Seeds powder (100g-200g) was well mixed with 2 mm diameter glass beads, and was then charged into the 0.5L extraction tube. The oil was extracted from the plant using supercritical CO₂ under above conditions.

Animal sample preparation: Fresh fat tissue with diatomaceous earth of animals (500g-600g which contained 100g-200g fat tissue) were charged into the 0.5L extraction tube. The oil was extracted from these animal fat tissues using supercritical CO₂ under above conditions.

(2) Methyl Esterification of oils

0.1g-0.2g seed oil was putted in 10mL tube and dissolved in 1.0 mL a solution, solved in petroleum ester and benzene (V/V=1:1), added 1.0 mL 0.1mol/L methanol-KOH solution, mixed with oscillation for 2 min and stilling for 15min, added 8.0 mL distilled water, static stratified, upper layer solution prepared for Gas Chromatographic injection.

(3) GS-MS Analysis of fatty acid compositions of oils

Instrument: GC-MS analysis was operated with a QP-5050A system (Shimadzu, Japan) by electron ionization with 70 eV, acceleration voltage at 1kv, scanning between 33 amu-500 amu and splitting time with 2.5min.

Chromatographic Condition: Column-a capillary column (0.25 mm i.d × 30 m, film 0.5 mm), HP-FFAP (Hewlett Packard, USA). Conditions-Injection volume: 0.2 μ L; Interface temperature: 255 °C; Injector temperature: 285 °C; Column temperature was initially 100 °C for 1 min, then raised to 255 °C at a rate of 10 °C /min, and finally held at 255 °C for 45 min; Helium gas was used as the carrier at a flow rate of 1.2 ml/min.

(4) Statistical analysis

An SPSS computer program was used. Hierarchical cluster analysis of fatty acid compositions (including C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:4, C20:5, C22:%, C22:6, SFA, MUFA, PUFA and UFA) was performed by the SPSS version 17.0.

3. Results and Discussion

3.1. The Results of Analysis on Fatty-Acid Compositions

The fatty-acid compositions of 23 plants oils and 16 animal oils were determined by GC-MS. The results were listed in Table I and their contents of saturated fatty acids(SFA), mono unsaturated fatty acids(MUFA), poly unsaturated fatty acids(PUFA) and unsaturated fatty acids were also calculated (listed in the same table).

3.2. Hierarchical Cluster Analysis of Fatty Acid Compositions

Hierarchical cluster analysis of fatty acid compositions (including including C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:4, C20:5, C22:5 and C22:6) was performed by SPSS. The dendrogram using average linkage (between oils) was shown in Fig. 1.

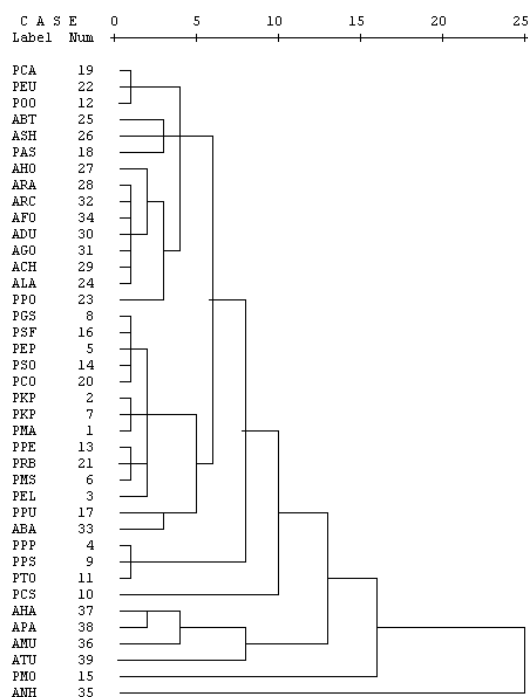


Fig. 1: Cluster analysis of oils by fatty acids.

Fig. 1 shows that ANH(*Nassarius hepaticus*) belongs to a single cluster as the highest content of C20:4(13.52%), oils of *Nassarius hepaticus* stands for C20:4; further can be found macadamia oil (PMO) for C16:1(16.0%); mullet Oil(AMU), hairtail oil (AHA), Pampus argenteus(APA) and turtle oil(ATU) for C20:5, C22:4 and C22:5; cottonseed oil(PCS) for C16(no more than 16 carbon chain); Purple Perilla(PPP), tung oil(PTO) and perilla seed(PPS) for C18:3; pumpkin seed(PPU) and badger oil(ABA) for C18; camellia Oil(PCA), *Eucommia ulmoides*(PEU) and Olive oil(POO) for C18:1. In this method, we can find more ideal resource of special fatty acid to further exploit.

AHO(horse oil), ARA(), ARC(rabbit oil), AFO(fox oil), ADU(duck oil), AGO(goose oil), ACH(chicken oil) and ALA(lard oil) are in the same cluster and from same kind-animal; The same rule can be inferred from this figure, PGS(grape seed oil), PSF(sunflower oil), PEP(Evening primose), PSO(soybean oil), PCO(corn oil), PKP(Korean pine), PMA(Manchurian Walnut), PPE(peanut oil), PRB(rice bran), PMS(Melon seeds) and PEL(Elderberry) in the same cluster, belong to plant. The different characteristics of fatty-acid compositions between animals and plant are found by this method because they belonged to different method. Thus animal and plant oil are different in fatty acids. These different oils should be further investigated to explain the difference between animals and plants in chemical components.

Note 1:Oils-Species-Manchurian Walnut(PMA), Korean Pine(PKP), Elderberry(PEL), Purple Perilla(PPP), Evening primose(PEP), Melon seeds(PMS), Korean pine2(PKP), grape seed oil(PGS); perilla seed(PPS), cottonseed oil(PCS), tung oil(PTO), Olive oil(POO), peanut oil(PPE), soybean oil(PSO), sunflower oil(PSF), pumpkin seed(PPU), macadamia oil(PMO), *Aquilaria sinensis*(PAS), camellia Oil(PCA),corn oil(PCO), rice bran(PRB), *Eucommia ulmoides*(PEU), palm oil(PPO), lard oil(ALA), beef tallow(ABT), sheep oil(ASH), horse oil(AHO), rabbit oil(ARA), chicken oil(ACH), duck oil(ADU), goose oil(AGO) , raccoon oil(ARC), badger oil(ABA), fox oil(AFO), *Nassarius hepaticus* (ANH), mullet Oil(AMU), hairtail oil (AHA), *Pampus argenteus*(APA), turtle oil(ATU).

Note 2: Abbreviation to oils, the first alphabet stands for oils resources from animals(A) or plants(P) .

Note 3: Components relative content were no more than 0.5% were not listed.

Note 4: SFA-saturated fatty acid; MUFA-mono unsaturated fatty acid; PUFA-poly unsaturated fatty acid; UFA-unsaturated fatty acid.

Note 5: C16 showed the relative content of those fatty acids which the carbon number is no more than 16.

Table 1: Fatty acid composition of oils

| Oils | Relative content of fatty acids/% | | | | | | | | | | | | SFA | MUFA | PUFA | UFA |
|------|-----------------------------------|-------|------|-------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | C16 | C16:1 | C18 | C18:1 | C18:2 | C18:3 | C20 | C20:1 | C20:4 | C20:5 | C22:5 | C22:6 | | | | |
| PMA | 3.43 | | 1.01 | 20.3 | 62.9 | 12.1 | | | | | | | 4.44 | 20.30 | 75.00 | 95.30 |
| PKP | 4.04 | | 2.01 | 29.7 | 47.5 | 14.6 | | | | | | | 6.05 | 29.70 | 62.10 | 91.80 |
| PEL | 15.2 | | 1.88 | 28.3 | 31.5 | 23.0 | | | | | | | 17.08 | 28.30 | 54.50 | 82.80 |
| PPP | 7.64 | | 2.78 | 20.2 | 15.1 | 54.3 | | | | | | | 10.42 | 20.20 | 69.40 | 89.60 |
| PEP | 7.15 | | 1.98 | 7.13 | 75.5 | 7.64 | | | | | | | 9.13 | 7.13 | 83.14 | 90.27 |
| PMS | 19.3 | | 8.06 | 28.8 | 41.8 | 1.20 | | | | | | | 27.36 | 28.80 | 43.00 | 71.80 |
| PKP | 4.64 | | 2.12 | 29.8 | 47.1 | 13.3 | | | | | | | 6.76 | 29.80 | 60.40 | 90.20 |
| PGS | 8.12 | | 3.01 | 18.21 | 69.5 | | | | | | | | 11.13 | 18.21 | 69.50 | 87.71 |
| PPS | 7.94 | | 2.48 | 19.3 | 15.4 | 54.5 | | | | | | | 10.42 | 19.30 | 69.90 | 89.20 |
| PCS | 3.85 | 2.10 | 1.75 | 50.2 | 20.5 | 7.54 | | 8.43 | | | | | 5.60 | 60.73 | 28.04 | 88.77 |
| PTO | 3.51 | | 3.64 | 6.82 | 7.53 | 72.3 | | | | | | | 7.15 | 6.82 | 79.83 | 86.65 |
| POO | 13.7 | | 2.51 | 71.1 | 10.0 | 0.63 | | | | | | | 16.21 | 71.10 | 10.63 | 81.73 |
| PPE | 11.6 | | 3.12 | 46.5 | 31.4 | 1.53 | | | | | | | 14.72 | 46.50 | 32.93 | 79.43 |
| PSO | 11.0 | | 4.21 | 23.4 | 53.2 | 7.82 | | | | | | | 15.21 | 23.40 | 61.02 | 84.42 |
| PMO | 11.8 | 16.0 | 5.55 | 58.6 | | | 3.73 | 2.64 | | | | | 21.08 | 77.24 | 0.00 | 77.24 |
| PSF | 6.81 | | 4.71 | 18.6 | 68.2 | 0.51 | | | | | | | 11.52 | 18.60 | 68.71 | 87.31 |
| PPU | 19.8 | | 22.5 | 25.3 | 30.5 | | | | | | | | 42.30 | 25.30 | 30.50 | 55.80 |
| PAS | 15.1 | | 16.6 | 65.5 | | | | | | | | | 31.70 | 65.50 | 0.00 | 65.50 |
| PCA | 8.75 | | 1.79 | 81.5 | 7.05 | | | | | | | | 10.54 | 81.50 | 7.05 | 88.55 |
| PCO | 12.8 | | 2.51 | 29.8 | 50.3 | 1.85 | | | | | | | 15.31 | 29.80 | 52.15 | 81.95 |
| PRB | 17.9 | | 2.21 | 47.3 | 30.6 | | | | | | | | 20.11 | 47.30 | 30.60 | 77.90 |
| PEU | 8.75 | | 1.79 | 81.5 | 7.08 | | | | | | | | 10.54 | 81.50 | 7.08 | 88.58 |
| PPO | 42.4 | | 3.00 | 43.3 | 11.2 | | | | | | | | 45.40 | 43.30 | 11.20 | 54.50 |
| ALA | 24.2 | 3.41 | 10.8 | 48.6 | 10.9 | 0.92 | | | | | | | 35.00 | 52.01 | 11.82 | 63.83 |
| ABT | 32.1 | 4.52 | 22.8 | 37.4 | 0.52 | | | | | | | | 54.90 | 41.92 | 0.52 | 42.44 |
| ASH | 28.2 | 5.01 | 14.6 | 49.6 | 0.73 | 1.25 | | | | | | | 42.80 | 54.61 | 1.98 | 56.59 |
| AHO | 30.5 | 6.47 | 4.54 | 35.1 | 7.19 | 15.8 | | | | | | | 35.04 | 41.57 | 22.99 | 64.56 |
| ARA | 36.0 | 7.65 | 6.43 | 28.8 | 10.2 | 8.79 | | | | | | | 42.43 | 36.45 | 18.99 | 55.44 |
| ACH | 20.2 | 4.10 | 7.15 | 44.9 | 14.5 | 0.82 | | | | | | | 27.35 | 49.00 | 15.32 | 64.32 |
| ADU | 24.2 | 3.50 | 6.43 | 48.8 | 16.3 | | | | | | | | 30.63 | 52.30 | 16.30 | 68.60 |
| AGO | 21.9 | 4.20 | 4.76 | 52.5 | 14.9 | 1.32 | | | | | | | 26.66 | 56.70 | 16.22 | 72.92 |
| ARC | 14.2 | 5.74 | 7.24 | 56.3 | 12.7 | 0.82 | | | | | | | 21.44 | 62.04 | 13.52 | 75.56 |
| ABA | | | 16.9 | 19.2 | 53.1 | 9.98 | | | | | | | 16.90 | 19.20 | 63.08 | 82.28 |
| AFO | 18.9 | 5.98 | 9.07 | 53.8 | 8.34 | | | | | | | | 27.97 | 59.78 | 8.34 | 68.12 |
| ANH | 20.2 | 1.17 | 15.8 | 5.01 | | | | 6.51 | 13.52 | 3.52 | 8.71 | 10.4 | 36.00 | 12.69 | 36.15 | 48.84 |
| AMU | 34.1 | 0.62 | 4.21 | 18.2 | 0.86 | | | 1.22 | | 5.64 | 1.82 | 4.37 | 38.31 | 20.04 | 12.69 | 32.73 |
| AHA | 29.3 | 4.90 | 7.18 | 27.8 | 0.58 | 0.64 | 0.58 | 0.72 | 0.75 | 4.72 | 2.58 | 17.3 | 37.06 | 33.42 | 26.57 | 59.99 |
| APA | 27.8 | 4.80 | 8.31 | 31.8 | | 0.52 | | | 1.12 | 4.21 | 4.59 | 11.3 | 36.11 | 36.60 | 21.74 | 58.34 |
| ATU | 37.0 | 14.6 | 5.24 | 25.7 | 2.85 | | | | 0.81 | 2.04 | | 2.40 | 42.24 | 40.30 | 8.10 | 48.40 |

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