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七种植物籽油中脂肪酸成分的比较分析

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摘　要:为寻求新的食用油资源,发展了一种快速可靠的气相色谱-质谱联用方法,用于植物籽油中脂肪酸成分的定性鉴定和含量测定。所建立的方法成功用于葡萄籽、南瓜籽和猕猴桃籽等七种植物籽油中的棕榈酸、十八烷酸、油酸、亚油酸和 α-亚麻酸的定性定量分析。结果表明,刺葡萄籽油、普通葡萄籽油、国外葡萄籽油、南瓜籽油、枸杞籽油和西番莲籽油均具有相似的脂肪酸谱,尽管其中它们所含上述五种脂肪酸含量不同,由于均存在人体所必需的饱和与不饱和脂肪酸,故可以用作替代食用油。猕猴桃籽油因为其存在高含量的 α-亚麻酸成分,可能是更好的食用油和营养油资源。本文首次对枸杞籽油、西番莲籽油和猕猴桃籽油脂肪酸成分进行绝对含量分析,为新的食用油资源的开发提供了重要的依据。

关键词:植物籽油;脂肪酸;气相色谱-质谱法;衍生化

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Comparative Analysis of Fatty Acid Composition in Seven Plant Seed Oils

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Abstract: To find new edible oil sources, a rapid and reliable gas chromatography mass spectrometry method for fatty acid identification and quantitation was developed. The proposed method was then used to quantitation of palmitic acid, stearic acid, oleic acid, linoleic acid and α-linolenic acid in seven different plant seend oils. The fatty acid profiles of spine grape seed, grape seed, foreign grape seed, pumpkin seed, barbary wolfberry fruit seed and passion flower seed oils are similar with different contents of the five fatty acids, indicating they can be alternative sources of edible oil due to the presence of all saturated and unsaturated fatty acids required for human health. Yangtao kiwifruit seed oil may be much better alternative source of edible and nutritional oil because of a high content of α-linolenic acid. This is the first research on the absolute content analysis of fatty acid composition of barbary wolfberry fruit seed oil, passion flower seed oil and yangtao kiwifruit seed oil which provides an important and scientific basis for new edible oil resource exploitation.

Key words: plant seed oil; fatty acid; gas chromatography-mass spectrometry; derivatization

Introduction

China is a large country in vegetable oil consumption. Soybean, rapeseed, peanut, sesame seed, and cottonseed are main resources of edible vegetable oil in China. But at present, nearly 60% of the total vegetable oil consumption needs to be imported because of the shortage of the above-mentioned resources. Thus, seeking new vegetable oil resources is a hotspot in the edible vegetable oil market of China as well as the whole world [1,2].

Grape seed (*Vitis vinifera* L.) is a worldwide well known oil seed crop containing 8%-15% oil. Matthaus stated that the production of oil from grape seeds could result in interesting edible oils with a comparable health benefit as olive oil. Grape seed oil is an extensive and important alternative edible oil resource with essential fatty acids. Therefore it is recognized that it's intake may be beneficial to prevent heart and circulato-

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ry problems [3]. While the main interest in grape seed oil lies in its high content of unsaturated fatty acids such as linoleic acid and α-linolenic acid [4]. For example, linoleic acid is the precursor of certain substances of physiological regulation. It can reduce blood cholesterol concentrations and the risk of many cardiovascular and cerebrovascular diseases (CCD), such as coronary artery disease, atherosclerosis, cerebral thrombosis, miocardial infarction, etc. α-Linolenic acid is the precursor of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Likewise, it can lower blood-lipid and blood pressure, inhibit platelet aggregation, and reduce thrombosis. As is well known, linoleic acid, α-linolenic acid, and arachidonic acid are three essential fatty acids that cannot be synthesized in the body. They can only be ingested from food. These fatty acids are of prime importance for health, and a number of abnormalities in polyunsaturated fatty acid (PUFA) profiles due to malnutrition or diseases have been reported [5]. From the nutritional and therapeutic point of view, the content of fatty acids (FAs) in grape seed oil is a key factor for their quality evaluation because of their important roles as metabolites and intermediates in biological processes [6-8].

Apart from grape seed oil, it was reported that some other vegetable seed oils such as sorghum [9], pumpkin seed [10,11], and sunflower seed [12] with essential fatty acids can also be used as alternative edible oil resources. In fact, vegetable seeds with essential fatty acids are so many more than these. For example, barbary wolfberry fruit seed, passion flower seed, and yangtao kiwifruit seed widely exist. Extraction and percentage composition analysis of the these three seed oils shows that they are promising alternative edible oil resources [13-18]. However, as to our knowledge, there is few report on the absolute amount analysis of their fatty acids so far.

The main objective of the present work is to develop a qualitative and quantitative method for the analysis of essential fatty acids in vegetable oil in order to seek new edible oil resources. This was done by comparative analysis of their fatty acids using supercritical carbon dioxide ($SC\text{-}CO_2$) extraction and derivatization gas chromatography mass spectrometry (GC-MS) method.

Experimental

Samples and reagents

Plant seed samples analyzed in this study were provided by Guangdong Academy of Agricultural Sciences, including grape (*Vitis vinifera* L.) seed, spine grape (*Vitis davidii* Foex.) seed, foreign grape (*Vitis vinifera* L.) seed, barbary wolfberry fruit (*Lycium barbarum* L.) seed, passion flower (*Passiflora incarnata* L.) seed, pumpkin (*Cucurbita pepo* L.) seed and yangtao kiwifruit (*Actinidia chinensis* Planch.) seed. They were identified by Professor Yang Guoping, who is working in The Third Hospital of Xiangya Medical College of Central South University, where the specimens were deposited.

All the plant seeds were crushed by a portable high speed grinder (Wenling LinDa Machinery Co. Ltd., Zhejiang, China) before extracting. The free fatty acid standards (palmitic acid, stearic acid, oleic acid, linoleic acid and α-linolenic acid, 99 + %) and the internal standard (heptadecanoic acid, 99 + %) were purchased from Sigma (St. Louis, MO, USA). HPLC grade methanol was from Hanbang Science and Technology Corporation (Jiangsu, P. R. China). Sulphuric acid and n-hexane were both supplied by Damao Chemical Reagent Factory (Tianjin, P. R. China). All other chemicals were of analytical reagent grade. Double-distilled water was deionized using Auto-double evaporator of Shensheng Science and Technology Corporation (Shanghai, P. R. China).

Supercritical carbon dioxide ($SC\text{-}CO_2$) extraction

Plant seed oil extraction was conducted in a HA231-50-06 semi-batch flow extraction apparatus produced by Nantong Hua'an Supercritical carbon dioxide extraction Corporation (Jiangsu, P. R. China). The extraction capacity was 1000 mL and a maximum flow rate of CO_2 was 50 Kg/h. The $SC\text{-}CO_2$ extraction conditions were as follows: extraction pressure: 30 MPa, separation temperature: 45 ℃, extraction time: 70 min. In all experiments, 100 g samples of powdered seeds were placed in the extractor cylinder, and filter mesh screens were placed at both ends of the cylinder to prevent any

carry over of particles. The cylinder was placed into the temperature-controlled chamber, and great care was taken to ensure that the air can purge through the filter before the extraction. The SC-CO_2 was pumped at a constant flow rate and directed to the bottom of the extractor for up-flow configuration. The supercritical phase from the extractor was passed through the valves, into which the pressure was throttled gradually and serially via two separators. The oil was collected every 15 min from the two separators and the CO_2 was cooled and recycled into the system. Successive collected samples were weighed and treated.

Derivatization of Sample

Direct transesterification of FAs is recommonded in FA analysis [19]. In the present work, a direct derivatization method, which can esterify both the lipid-bound and free fatty acids (FFAs), was developed mainly based on the literature [20] with minor revision. The derivatization approach is as follows: Equal volumes (10 μL) of heptadecanoic acid (C17∶0) internal standard (3 mg/mL in n-hexane; stored at - 40 ℃) was added to 0.5 μL of each sample. After that, 2.0 mL of 1% H_2SO_4-CH_3OH was added, vortex-mixed for 30 s and reacted at 80 ℃ water bath for 60 min. Then, the lipids were extracted with 2.0 mL of n-hexane twice using a vortex mixer for 30 s. Samples containing the fatty acid methyl esters were blown to dryness under nitrogen, dissolved in 1.0 mL of n-hexane, and stored at 4 ℃ away from light until GC analysis.

Gas chromatography mass spectrometry

Analyses were carried out in a Hewlett-Packard 6890 gas chromatograph fitted with a DB-23 capillary column (30 m × 0.25 mm i. d., film thickness 0.25 μm), interfaced with an Hewlett-Packard mass selective detector 5973N (Agilent Technologies, USA) operated by HP Enhanced ChemStation software, G1701DA MSD ChemStation Rev. D. 00. 00. 38. Oven temperature program: 80 ℃ to 175 ℃, at 25 ℃/min, then programmed to 220 ℃, at 4 ℃/min, and held for 5 min at 220 ℃; injector temperature: 250 ℃; carrier gas: helium, adjusted to a column velocity of flow 1.0 mL/min, splitting ratio 20∶1, interface temperature: 250 ℃, standard electronic impact (EI) MS source temperature: 230 ℃; MS quadrupole temperature: 150 ℃, mass scan range: 30-500 units, scan velocity: 3.12 scans/s. The identification of fatty acid methyl esters (FAMEs) was carried out by searching in NIST 02 mass database as well as the mass spectrum and retention time of authentic compound.

Validation of the analytical method

The standard stock solutions of fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, and α-linolenic acid) and internal standard stock solution of heptadecanoic acid (C17∶0) were prepared in n-hexane. The working solutions were obtained by further dilution with n-hexane. All solutions were stored at 4 ℃ away from light before analysis. Accuracy was assessed using standard addition method at three different (low, median and high) concentration levels of standard compounds by replicate measurements (n = 6). In order to determine the accuracy of the method, Sample 7 was spiked with a known amount of the five standard fatty acid compounds, processed under the proposed transesterification process and analyzed using the specified GC-MS method. The recovery rates were obtained by the ratios of the found contents to the sum of original and added contents.

The analytical precision (each, n = 6) were evaluated by analysis of all the samples at different times during the same day. The concentration of each sample was determined using calibration standards prepared on the same day.

Results and Discussion

Optimization of the derivatization procedure and GC-MS analysis

The derivatization procedure of fatty acids was determined mainly based on the literature [20]. But three factors such as derivatization temperature, derivatization time, and the amount of derivatization reagent were optimized by single factor test to obtain higher precision. Yangtao kiwifruit seed oil (Sample 7) is taken as an example to illustrate the derivatization condition optimization result obtained. Fig. 1 shows the concentration of fatty acid methyl esters (FAMEs) obtained at different derivatization time (10, 20, 30, 60, 90 min), tempera-

ture (40,60,80,100 ℃) and amount of derivatization reagent (0.5,1.0,2.0,3.0mL).

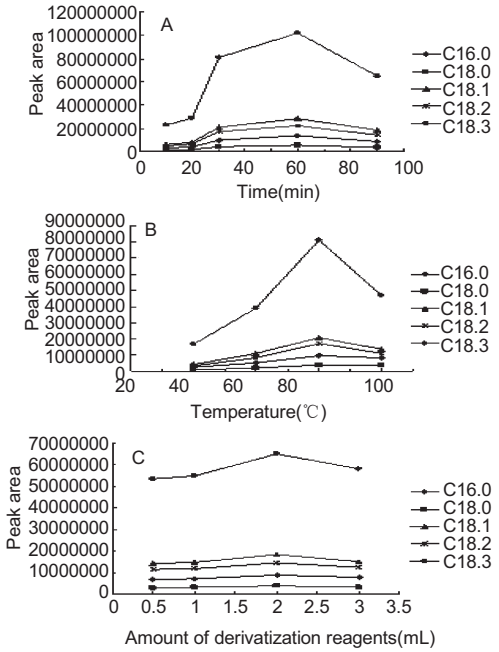

Fig. 1 The derivatization profiles for fatty acids in Sample 7

(a) The derivatization time profiles; (b) The derivatization temperature profiles; (c) The amount of derivatization reagents profiles.

From Fig. 1, one can find that the concentration of FAMEs were usually increased with the increase of derivatization time, temperature and amount of derivatization reagent. But that of α-linolenic acid methy ester was decreased obviously when the derivatization time last longer than 60 min or the derivatization temperature was higher than 80 ℃. This may because of the decomposition of α-linolenic acid methy ester[21]. Thus the optimum derivatization time, temperature and amount of derivatization reagent can be determined to be 60 min, 80 ℃ and 2 mL, respectively. And all the subsequent experiments were carried out using 2 mL H_2SO_4-CH_3OH (1%, v/v) solution at 80 ℃ for 60 min.

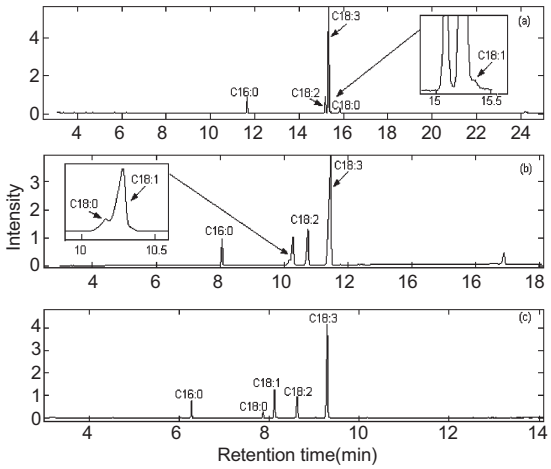

Fig. 2 GC-MS profiles of Sample using different columns

(a) HP-5: oven temperature program: 150 ℃ to 280 ℃, at 4 ℃/min; FFAP: oven temperature program: 80 ℃ to 150 ℃, at 15 ℃/min, then programmed to 180 ℃, at 3 ℃/min, and held for 2 min at 180 ℃, heated at 15 ℃/min to 220 ℃, then held for 5 min at 220 ℃; (b) DB-23: oven temperature program: 80 ℃ to 175 ℃, at 25 ℃/min, then programmed to 220 ℃, at 4 ℃/min, and held for 5 min at 220 ℃

To improve the separation of the FAMEs, three capillary columns (HP-5, FFAP and DB-23) were employed and Sample 7 was also taken as an example to illustrate the process. Fig. 2 shows the separation results in the three cases, from which it is shown that when a DB-23 column was used, the best resolution was obtained within a few minutes (Fig. 2 (c)). Therefore, DB-23 column was selected for the GC-MS analysis of the FAMEs of plant seed oils.

Method validation

The regression equations, correlation coefficients (R), linear ranges and limits of detection (LOD) of the five fatty acids were listed in Table 1. Good linear relationships (R = 0.9997 to 0.9999) between peak areas and the concentrations, wide linear ranges and low LODs

Table 1 Calibration curves, orrelation coefficient, linear ranges and detection limits of the fatty acids

Fatty acids	Calibration curve	Correlation coefficient/R	Linear range (ng/mL)	LOD[a] (ng/mL)
Palmitic acid, 16:0	y = 62.387x - 0.0670	0.9999	4.50-900	9.0
Stearic acid, 18:0	y = 68.252x - 0.9732	0.9999	4.52-904	9.0
Oleic acid, 18:1	y = 56.572x + 0.1035	0.9997	4.55-910	9.1
Linoleic acid, 18:2	y = 54.799x + 0.8852	0.9997	4.51-902	9.0
α-Linolenic acid, 18:3	y = 49.923x - 0.5381	0.9997	4.57-914	9.1

Note: y and x represent peak area and concentration in ng/mL of fatty acid; LOD: limit of detect.

Table 2 Recoveries of fatty acids determined by standard addition method (n = 6)

Fatty acids	Original concentration (ng/mL)	Added concentration (ng/mL)	Detected concentration (ng/mL)	Recovery (%)	RSD (%)
Palmitic acid	27.2	9.00	33.5	92.5	4.1
	27.2	27.0	51.3	94.6	3.4
	27.2	45.0	72.0	98.4	2.2
Stearic acid	24.9	9.04	31.7	93.4	3.1
	24.9	27.1	49.8	95.8	4.6
	24.9	45.2	65.6	93.6	2.8
Oleic acid	49.0	9.10	55.7	95.9	2.6
	49.0	45.5	90.0	95.2	3.8
	49.0	91.0	137.1	97.9	4.3
Linoleic acids	18.5	9.02	25.15	91.4	5.6
	18.5	45.1	60.6	95.3	2.8
	18.5	90.2	105.1	96.7	3.5
α-linolenic acid	74.5	45.7	114.0	94.8	4.2
	74.5	91.4	161.2	97.2	3.1
	74.5	182.8	244.7	95.1	2.7

Note: Recovery (%) = [Detected concentration/(Original concetration + Added concentration)] × 100

were exhibited. The recoveries (in %) of the five fatty acids from Sample 7 were detected by standard addition method at low, median and high concentrations. The recovery percents were in the ranges of 91.4% to 98.4% as listed in Table 2. These values were within the limits of acceptable variability for the analyte concentration of these kinds of samples.

Identification and content comparative analysis of fatty acids in plant seed oils

The seven different plant seed oils described above were analyzed by the proposed method. The chromatograms of the FAMEs profiles obtained are shown in Fig. 3. The major fatty acids were identified by comparison of their mass spectra with NIST02 mass database through G1701DA mass spectrum ChemStation and as well as by comparison of their mass spectra and retention times with those of the injecting authentic components at the same conditions. The five common peaks in the seven plant seed oils were identified to be palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and α-linolenic (C18:3) acid. They are essential fatty acids for health. The relative amounts of unsaturated fatty acids (UFAs) and saturated fatty acids (SFAs) in the plant seed oils were presented on the basis of free fatty acids according to peak area normalization method (Table 3). From Table 3, it can be seen that, the total relative amounts of UFAs and SFAs were in the ranges of 53.9% to 74.1% and 11.1% to 23.0%, respectively. The distribution of fatty acid composition was quite similar except yangtao kiwifruit seed oiL. Yangtao kiwifruit seed oil has a high percentage of α-linolenic acid (43.7%) and a low content of linoleic acid (10.1%) as well as adequate content of oleic acid (14.2%), palmitic acid (7.4%), and stearic acid (3.3%). Meanwhile, α-linolenic acid was not detected in other samples except passion flower seed (0.5%) and pumpkin seed (0.6%) with extremely

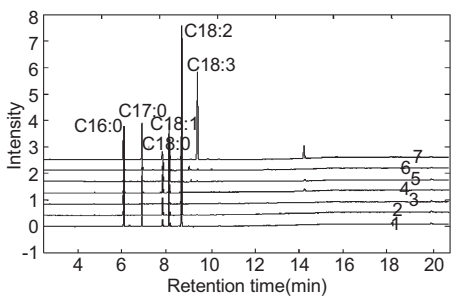

Fig. 3 GC-MS profiles of the seven seed oils

1. Spine grape seed; 2. Grape seed; 3. Foreign grape seed; 4. Barbary wolfberry fruit seed; 5. Passion flower seed; 6. Pumpkin seed; 7. Yangtao kiwifruit seed.

Table 3 Fatty acid identified and their relative amount of different seed oils

Sample ID	SFAs (%)[a]			UFAs (%)[a]			
	Palmitic acid	Stearic acid	Totol	Oleic acid	Linoleic acid	α-Linolenic acid	Total
1	11.7	3.8	15.5	11.9	43.7	ndb	55.6
2	10.4	4.7	15.1	11.5	56.5	nd	68.0
3	7.4	3.7	11.1	17.5	44.2	nd	61.7
4	17.2	5.8	23.0	19.7	34.2	nd	53.9
5	12.6	3.3	15.9	12.5	58.0	0.5	71.0
6	8.7	4.1	12.8	21.8	51.7	0.6	74.1
7	7.4	3.3	11.7	14.2	10.1	43.7	68.0

1. Spine grape seed; 2. Grape seed; 3. Foreign grape seed; 4. Barbary wolfberry fruit seed; 5. Passion flower seed; 6. Pumpkin seed; 7. Yangtao kiwifruit seed. a: The amount of UFAs and SFAs in oil on the basis of free fatty acids; b: Not detected.

low percentages of α-linolenic acid. As for Samples 1 to 6, linoleic acid was shown to be the most important component ranged from 34.2% to 58%, followed by oleic acid (11.5%-21.8%) and palmitic acid (7.4%-17.2%), though some minor difference exists between the percentages of the major fatty acids from each other.

To evaluate their quality, the comparsion of absolute content of fatty acids in different plant seed oils seems more reasonable and important. Thus our developed quantitation method was used to determine the contents of the fatty acids in the seven different plant seed oils quantitatively. Table 4 shows the summary results obtained by using the calibration curve of each investigated compound. From Table 4, it is found that the differences of contents of palmitic acid, stearic acid, and oleic acid among the seven samples were not obvious. But the contents of linoleic acid ranged from 75 to 628 milligram per milliliter of oil, the maximum difference was about 8 times. The highest content of linoleic acid was detected in the passion flower seed oil (Sample 5). While α-linolenic acid was not detected from Samples 1 to 4 and its content in Samples 5 and 6 was actually very low. The highest content of α-linolenic acid was observed in the yangtao kiwifruit seed (Sample 7), in which the content of α-linolenic acid (0.294 g/mL) was much higher than linoleic acid (75 mg/mL). The relative standard deviations (RSDs) listed in Table 4 were less than 5.0% except for that (5.8%) of α-linolenic acid in Sample 6 because of its low content.

Table 4 Contents of fatty acids in the seven seed oils

Sample ID	Palmitic acid (g/mL)	RSD (%)	Stearic acid (g/mL)	RSD (%)	Oleic acid (g/mL)	RSD (%)	Linoleic acid (g/mL)	RSD (%)	α-linolenic acid (g/mL)	RSD (%)
1	0.195 ± 0.039[a]	3.8	0.119 ± 0.016	1.2	0.162 ± 0.023	1.3	0.269 ± 0.081[a]	3.0	nd[b]	nd
2	0.186 ± 0.034	1.7	0.138 ± 0.035	2.4	0.150 ± 0.056	3.5	0.529 ± 0.040	0.7	nd	nd
3	0.114 ± 0.023	2.0	0.110 ± 0.020	1.8	0.231 ± 0.025	0.9	0.251 ± 0.021	0.8	nd	nd
4	0.285 ± 0.026	0.8	0.198 ± 0.027	1.2	0.250 ± 0.042	1.5	0.330 ± 0.051	1.5	nd	nd
5	0.207 ± 0.031	1.3	0.103 ± 0.024	2.2	0.169 ± 0.029	1.5	0.628 ± 0.051	0.8	0.004 ± 0.001	1.9
6	0.132 ± 0.044	3.2	0.125 ± 0.013	1.0	0.263 ± 0.051	1.7	0.467 ± 0.084	1.8	0.005 ± 0.004	5.8
7	0.110 ± 0.032	2.8	0.100 ± 0.023	2.2	0.198 ± 0.039	1.9	0.075 ± 0.021	2.8	0.294 ± 0.028	0.9

1. Spine grape seed; 2. Grape seed; 3. Foreign grape seed; 4. Barbary wolfberry fruit seed; 5. Passion flower seed; 6. Pumpkin seed; 7. Yangtao kiwifruit seed. [a]Data presented are in means ± standard deviation, $n = 6$; [b]not detected.

Conclusion

This is the first research on the fatty acid compositions of barbary wolfberry fruit seed oil, passion flower seed oil, and yangtao kiwifruit seed oil, which provides an important and useful scientific basis for new edible oil resource exploitation.

(1) A derivative gas chromatography mass spectrome-

try method was developed for fatty acid analysis in plant seed oil after optimizing of derivative and chromatographic conditions. The method was validated to be precise and reliable with low detecting limit and wide linear range having high correlation coefficients ranging from 0.9997 to 0.9999 of palmitic, stearic, oleic, linoleic acid, and α-linolenic acid.

(2) Seven different plant seed oils including spine grape seed, grape seed, foreign grape seed, pumpkin seed, barbary wolfberry fruit seed, passion flower seed, and yangtao kiwifruit seed oils were analyzed. Their total relative amounts of unsatured fatty acids and saturated fatty acids were in the ranges of 53.9% to 74.1% and 11.1% to 23.0%, respectively. The contents of palmitic, stearic, oleic, and linoleic acid in the first six samples ranged from 0.114 to 0.285 g/mL, 0.110 to 0.198 g/mL, 0.150 to 0.263 g/mL, and 0.251 to 0.628 g/mL, respectively.

(3) The first six plant seed oils can be used to be alternative sources of edible oil due to the presence of all saturated and unsaturated fatty acids required for human health. Being different from the others, yangtao kiwifruit seed oil has high content of α-linolenic acid (0.294 g/mL) and relative low content of linoleic acid (0.075 g/mL) and certain saturated fatty acids, it may be much better alternative source of edible and nutritional oil because α-linolenic acid and linoleic acid are two main functional constituents.

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