Analysis of the Fatty Acid Composition in Grape Seed

by GC / MS

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Abstract Fatty acids composition in grape seed are analyzed． Soxhlet extraction method toextract the fatty oil in the grape seed oil，with potassium hydroxide methanol solution to methyl esterof fatty acid in fatty oil，and gas chromatography-mass spectrometry instrument is analyzed． The results showthat 6 fatty acids are detected in the mountain grape seed，the major fatty acids are linoleic acid and oleic acid．

Key words Grape;Atty acid;Soxhlet extraction;GC / MS.

The grape (scientific name: Vitis vinifera L.)is Grape family, Grape genus, Woody vines plant. Grapes are rich in protein, carbohydrates, minerals and a variety of vitamins. Raw food tastes sweet and delicious, rich in savory sauce, is a delicious mountain wild fruit [1-4]. Grapes are the raw material for making wines. The wines are rich in crimson wines and are very good in flavor. They are good drinks. Grapeseed is a by-product of grape wine production. The study of fatty acid composition in grape seed is rarely reported. In order to make better use of the by-products produced from the processing of grape wine, realize the recycling of resources. In this paper, Soxhlet extraction method was used to extract the grape seeds and the fatty acids in the extracted fatty oil were methylated with potassium hydroxide and methanol solution. The main chemical components of the derivative products were separated and identified by gas chromatography-mass spectrometry. Provide a certain scientific basis for the development and utilization of grape seed.

1 Experimental section

1.1 Instruments, reagents, and samples

instrument: Agilent Technologies Gas Chromatograph-Mass Spectrometer (US Hewlett Packard). 80-2B Desktop Centrifuge (Shanghai Anting Scientific Instrument Factory); Soxhlet Extractor,; RE-52C Rotary Evaporator (Shanghai Yarong Shenghua Instrument Factory); HX-200A High-speed Chinese medicine grinder.

Reagents: petroleum ether (60 ~ 90 °C), n-hexane, methanol, potassium hydroxide, anhydrous sodium sulfate (all analytical grade).

Sample: grape seed was collected from Sariwon Grape Farm, Democratic People's Republic of Korea. The sample was crushed by a high-speed Chinese medicine grinder and sieved (pore size 0.4 mm) for use.

1. 2 Fat oil extraction

Precision weighing 1.1 processed samples 5.00 g into the filter paper tube, then add to the Soxhlet extractor and add 150 m L of petroleum ether, recirculate in a 70 °C water bath for 6 h, the pale yellow transparent extract was obtained, concentrate under reduced pressure, the fat oil yield is 19. 6%.

1. 3 The methyl esterification of fatty acids [5 - 7]

At 1. 2 in the extracted fatty oil, add 8 m L of n-hexane, 0. 5 m L/L potassium hydroxide methanol solution 2 m L, in the 70 °C water bath reflux 20 min, cooling, adding 12 m L distilled water, ultrasonic 5 min, the solution was centrifuged at 3 000 r / min for 10 min.The supernatant was removed, dried over anhydrous sodium sulfate and centrifuged again. The supernatant was subjected to GC/MS analysis.

1. 4 Gas chromatography-mass spectrometry conditions

1. 4. 1 chromatographic conditions

Column: Agilent 19091S-433UI;HP-5ms Ultra Inert; -60 °C—325 °C (350 °C): 30 m x 250 μm x 0.25 μm, (initial value) 80 °C

Pressure 9.3825 psi,Flow rate 1 mL/min,Average line speed 36.966 cm/sec,Residence time 1.3526 min,Flow program open,Flow program 1 mL/min for 0 min,Run time 26 min.

1. 4. 2 Mass spectrometry conditions

The ion source is an electron bombardment source; the ion source temperature is 325 °C; the ionization voltage is 70 eV; the electron multiplier voltage is 1 988 V; the emission current is 34. 6 μA; interface temperature 325 °C; mass range 20 to 500 m/z.

1. 5 experimental methods

Take 1. 3 sample solution 0. 4 μL for experimentation. Retrieving the NIST98 spectral library from the G1701BA ChemStation data processing system. And compared with the fifteen peak index and the standard spectrum of the EPA/NIH mass spectrum atlas respectively, determine the individual chemical components in the sample [8-9]. Then we use area normalization method for quantitative analysis, obtain the relative percentage of each chemical component in the sample.

2 Results and analysis

The sample was analyzed and identified by a GC/MS analyzer, the total ion chromatograms of grape seed fatty acid methyl esters and their chemical constituents are shown in Figure 1.

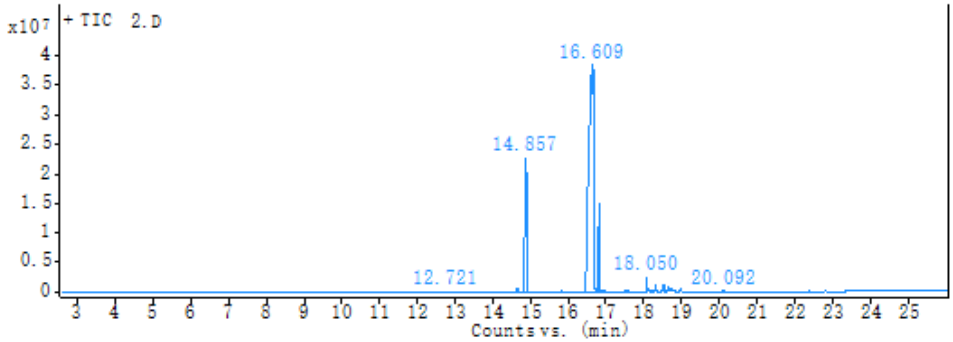

Fig. 1 Total ion chromatogram of fatty acid methyl esters and various chemical components of grape seed

The relative percentages of the chemical composition of the fatty acid methyl esters and various chemical components in the sample are given in Table 1,2.

As can be seen from Table 1,2 acorrding keep time,a total of 15 chemical constituents were detected after the methyl esterification of the grape seed. In these, chemical constituent for keep time (16.609min) is the highest , next constituents for keep time (16.774min and 14.857 min) are higher than other. major chemical constituents are unsaturated fatty acids (Linoleic acid, Linolenic acid)and saturated fatty acids(Hexadecanoic acid,Stearic acid). For them, specific analysis results are given in Fig 2,3,4 and Table 3,4,5 .

Table 1 Chromatogram peak list

No	Keep time	Peak height	Peak height percentage	Area	Area percentage	Area plus percentage	Symmetry factor	Width
1	12.721	228375.21	0.59	361102.18	0.1	0.08	1.16	0.147
2	14.615	520407.91	1.35	813948.85	0.23	0.18	1.2	0.073
3	14.857	22917464.26	59.65	53865074.8	14.92	11.59	0.28	0.164
4	16.609	38419670.13	100	361096467.8	100	77.67	0.33	0.3
5	16.774	15170570.93	39.49	27298041.4	7.56	5.87	0.45	0.129
6	16.915	340374.54	0.89	1659231.49	0.46	0.36	5.14	0.229
7	17.492	451655.09	1.18	734863.08	0.2	0.16	1.33	0.123
8	18.05	2603161.99	6.78	3978770.82	1.1	0.86	1.39	0.058
9	18.086	595322.51	1.55	1038357.79	0.29	0.22	3	0.047
10	18.15	227977.77	0.59	650395.01	0.18	0.14	1.17	0.071
11	18.274	1297890.5	3.38	3131677.01	0.87	0.67	0.47	0.154
12	18.48	1299636.37	3.38	3173891.39	0.88	0.68	1.22	0.124
13	18.603	794342.35	2.07	5794191.44	1.6	1.25	2.88	0.276
14	18.939	466692.5	1.21	971099.95	0.27	0.21	0.82	0.124
15	20.092	223244.99	0.58	372691.64	0.1	0.08	0.9	0.094

Table 2 Compound table

No	Compound label	Keep time	name	Molecular formula	MFG Molecular formula	DB Molecular formula
1	Cpd 1: Methyl tetradecanoate	12.721	Methyl tetradecanoate	$C_{15}H_{30}O_2$	$C_{15}H_{30}O_2$	$C_{15}H_{30}O_2$
2	Cpd 2: 9-Hexadecenoic acid, methyl ester, (Z)-	14.615	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	$C_{17}H_{32}O_2$	$C_{17}H_{32}O_2$
3	Cpd 3: Hexadecanoic acid, methyl ester	14.857	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	$C_{17}H_{34}O_2$	$C_{17}H_{34}O_2$
4	Cpd 4: 8,11-Octadecadienoic acid, methyl ester	16.609	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	$C_{19}H_{34}O_2$	$C1_9H_{34}O_2$
5	Cpd 5: Methyl stearate	16.774	Methyl stearate	$C_{19}H_{38}O_2$	$C_{19}H_{38}O_2$	$C_{19}H_{38}O_2$
6	Cpd 6: 10,13-Octadecadienoic acid, methyl ester	16.915	10,13-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	$C_{19}H_{34}O_2$	$C_{19}H_{34}O_2$
7	Cpd 7: Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	17.492	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	$C_{20}H_{38}O_2$	$C_{20}H_{38}O_2$	$C_{20}H_{38}O_2$
8	Cpd 8: E,E,Z-1,3,12-Nonadecatriene-5,14-diol	18.05	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	$C_{19}H_{34}O_2$	$C_{19}H_{34}O_2$
9	Cpd 9: Oxiraneoctanoic acid, 3-octyl-, methyl ester	18.086	Oxiraneoctanoic acid, 3-octyl-, methyl ester	$C_{19}H_{36}O_3$	$C_{19}H_{36}O_3$	$C_{19}H_{36}O_3$
10	Cpd 10: 6,9,12-Octadecatrienoic acid, methyl ester	18.15	6,9,12-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	$C_{19}H_{32}O_2$	$C_{19}H_{32}O_2$
11	Cpd 11: cis-13-Eicosenoic acid, methyl ester	18.274	cis-13-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	$C_{21}H_{40}O_2$	$C_{21}H_{40}O_2$
12	Cpd 12: Methyl 18-methylnonadecanoate	18.48	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	$C_{21}H_{42}O_2$	$C_{21}H_{42}O_2$
13	Cpd 13: .gamma.-Sitosterol	18.603	.gamma.-Sitosterol	$C_{29}H_{50}O$	$C_{29}H_{50}O$	$C_{29}H_{50}O$
14	Cpd 14: 6,9,12-Octadecatrienoic acid, methyl ester	18.939	6,9,12-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	$C_{19}H_{32}O_2$	$C_{19}H_{32}O_2$
15	Cpd 15: Docosanoic acid, methyl ester	20.092	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	$_{C23}H_{46}O_2$	$C_{23}H_{46}O_2$

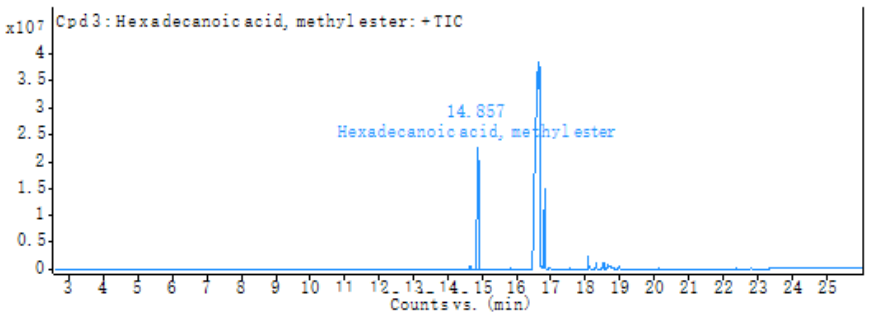

Fig.2-1 Chromatogram of chemical constituent for keep time (14.857min)

Table 3 Spectrum peak list

m/z	z	Abundance
41.1		341667.63
43.1	1	488960.64
55.1	1	533757.84
69.1	1	348709.55
74.1	1	2685399.25
87.1	1	1962618.86
143.1	1	779168.34
227.2	1	775871.45
239.2	1	639774.64
270.2	1	880467.91

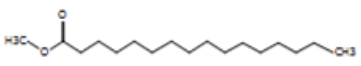

Fig.2-2 Compound structure of chemical constituent for keep time (14.857min)

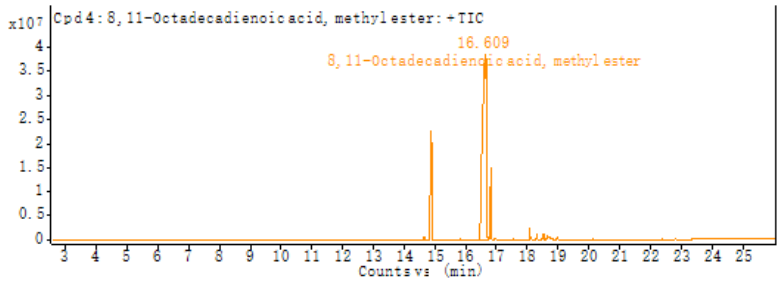

Fig. 3 -1 Chromatogram of chemical constituent for keep time (16.609min)

Table 4 Spectrum peak list

m/z	z	Abundance
41.1	1	5215.73
55.1	1	7734.28
67.1		9349.25
69.1	1	4845.22
74.1	1	5240.98
79.1		4435.23
81.1		8533.38
82.1		4850.85
95.1		6737.77
96.1		5170.77

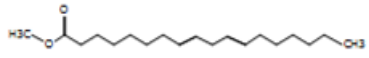

Fig. 3-2 Compound structure of chemical constituent for keep time (16.609nm)

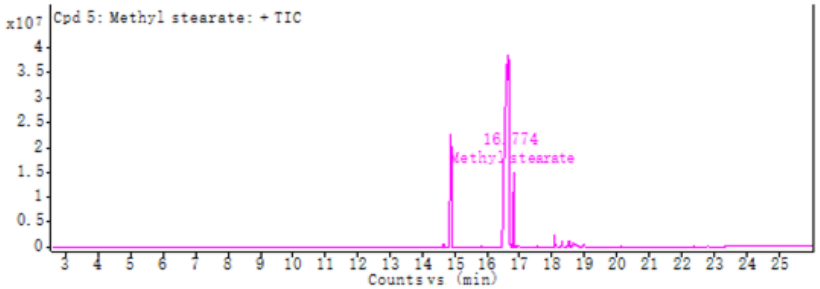

Fig. 4 -1 Chromatogram of chemical constituent for keep time (16.774nm)

Table 5 Spectrum peak list

m/z	z	Abundance
43.1	1	327677.5
55.1	1	350864
69.1	1	240111
74.1	1	1652310.75
87.1	1	1188278
143.1	1	508590
199.1	1	279047.88
255.2	1	532749.25
267.2	1	334785.38
298.2	1	740311.13

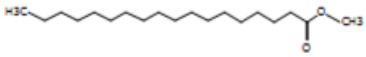

Fig. 4-2 Compound structure of chemical constituent for keep time (16.774min)

Linoleic acid content is 77.67%, Hexadecanoic acid content is 11.59%, Stearic acid content is 5.87%,are more higher than other fatty acid contents .

3 Discuss

From the above experimental results, it can be seen that the main chemical constituents of grape seed fatty acids are unsaturated fatty acids. Studies have shown that unsaturated fatty acids are fatty acids that cannot be synthesized and are essential in the human body. They have the functions of relieving excess cholesterol in the

blood, enhancing cell membrane permeability, preventing myocardial tissue and atherosclerosis [10]. How much of the body's intake of unsaturated fatty acids can also directly affect the synthesis of prostaglandin, has many effects on the body. Among them, the lack of linoleic acid causes cholesterol to bind to some saturated fatty acids and cause metabolic disorders. Grape seed has high nutritional value and is a natural health food with great development potential.

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