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Determination of Fatty Acid, C, H, N and Trace Element Composition in Grape Seed by GC/MS, FTIR, Elemental Analyzer and ICP/OES

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Abstract: Four different methods were used for the investigation of fatty acid, C, H, N and trace element composition of grape seed oil and pulp. The fatty acid profile of the grape seed oil was measured by GC/MS. Moreover, structural analysis of the oil was carried out by FT/IR spectrometry with a Zn Se ATR accessory. The quantification of selected metals Na, K, Mg, Ca, Mn, Fe, Zn and Cu in grape seed oil and pulp was carried out using microwave assisted digestion followed by ICP/OES. Grape seed was analyzed for C, H and N by elemental analyzer.

Key words: Grape seed, GC/MS, FTIR, ICP/OES

Üzüm Çekirdeğinde, GC/MS, FT/IR, Elementel Analiz Cihazı ve ICP/OES ile Yağ Asidi, C, H, N ve Eser Element Bileşiminin Belirlenmesi

Özet: Üzüm çekirdeği ve posasında dört farklı yöntem kullanılarak yağ asidi, C, H, N ve eser element bileşimi incelenmiştir. Üzüm çekirdeği yağının yağ asidi profili, GC/MS kullanılarak belirlenmiştir. Ayrıca, yağın yapı analizi, FT/IR spektrometre cihazı Zn Se ATR aparatıyla kullanılarak gerçekleştirilmiştir. Üzüm çekirdeği yağında ve posasında seçilen metallerin Na, K, Mg, Ca, Mn, Fe, Zn ve Cu analizi, numunelerin mikrodalga sisteminde hazırlanmasından sonra ICP/OES sistemi kullanılarak gerçekleştirilmiştir. Üzüm çekirdeğinin C, H ve N analizi, element analiz cihazında yapılmıştır.

Anahtar Kelimeler: Üzüm çekirdeği, GC/MS, FTIR, ICP/OES

1. Introduction

Grapes are major fruit yields and about 80% of the harvest is used by the wine making industry [1]. Grape seeds are around 15% of the solid waste produced in wine industries. The grape seeds are fractionated and pressed in order to remove the oil after fermentation and dehydration. Oil content of grape seeds strongly depends on grape variety, though the usual range is 10–16% of dry weight. Also, grape seed oil is contain tannins, unsaponifiable lipids, triglycerides, fatty acids and estrols. The antioxidant properties of these compounds makes grape oil very resistant to peroxidation. And grape seed oil is using as cosmetic industry, culinary, pharmaceutical and medical purposes [2,3]. The high content in polyunsaturated fatty acids (PUFAs) makes it a high-quality nutritional oil. Owing to this high PUFAs content, it exhibits properties prevention of thrombosis, inhabitation of cardiovascular diseases, reduction of cholesterol in serum, dilation of blood vessels, redeuction cancer and regulation of autonomic nerves [1, 4]. The ratio of monosaturated fatty acids (MUFA), PUFAs and saturated fatty acids (SFA) of grape seed oil were 29,84%, 53,01% and 16,7% respectively. Two types of PUFA n-6 (linoleic) and n-3 (linolenic), are important with

regard to health, disease and stability of grape seeds [2]. The ratios of PUFA/SFA was 3,17 in grape seed. In addition, linoleic acid was prodominant fatty acid (52,26%) and followed by oleic acid (29,1%). In the chemical composition of grape seed oil the following fatty acids are found: palmitic acid 7,2-8,5%, stearic acid 3,8-3,9%, oleic acid 15,4-15,6%, linoleic acid 71,7-73,1%, linolenic acid 0,3-0,6% [4,5]. In the C, H and N composition of grape seed oil are found: C 50,14%, H 7,07%, N 1,94% [6]. The determination of inorganic profile of grape seed oil is important. Because some elements have been shown metabolic role in the human health and wine quality [7]. The determination of metals in wine has been considered to be of great interest. Because it allows the definition of a "fingerprint" for each of them that permits one to verify the certified brand of origin (CBO) [8].

Alternative methods for oil extraction have been proposed, as hot water extraction, soxhlet extraction, superheated hexane extraction and supercritical fluid extraction (SFE) [7,8]. Analysis of fatty acid composition and volatile compounds of edible oil by chromatographic techniques (HS/GC-FID, GC/MS) [1, 11, 12, 13], spectroscopic techniques (FTIR, Raman, NMR) [14, 15, 16, 17, 18, 19, 20, 21]. Most of the papers published in the metal analysis make use of atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry/optical emission spectrometry (ICP-AES/OES) [7, 8, 22, 23, 24].

The purpose of this work is to evaluate the fatty acid, C, H, N and trace elemental composition of grape seed oil using gas chromatography-mass spectrometry (GC/MS), fourier transform infrared spectroscopy (FT/IR), elemental analyzer and ICP/OES.

2. Experimental

2.1. Reagents

n-Hexane (Merck, Darmstadt, Germany), standards of fatty acid methyl esters (FAME) (Supelco 37 Component FAME Mix, Supelco, Bellefonte, PA) were used.

For the AAS work nitric acid (65 wt. % p.a.), hydrogen peroxide (30 wt. % p.a.) and single element standards (Merck, Dermstadt, Germany) were used.

2.2. Sample Preparation

Grape seeds were obtained from a herbalist in Isparta (Turkey). They were a mixture of several grape varieties preserved below −20°C until use. Seeds were dried for 4 hour at 110°C. Then, they were milled using a Heidolph Diax 9000 (Burladingen, Germany) and kept in a desiccator until use.

2.3. Fatty Acid Extraction

The oil extraction was using Soxhlet extraction system and hexane as solvent. An extraction time of 8 h was chosen. With this aim, four extractions were carried out using 100 ml n-hexane and 3 g of smallest particle size milled seeds. After extraction for 8 h (80°C), the extracts were evaporated. by rotary vacuum evapora evaporation (Laborota

4001, Germany) at 40°C and the oil was methylated dissolved in 1 ml of hexane prior to injection into the gas chromatograph [25,26].

2.4. Preparation of Fatty Acid Methyl Esters (FAMEs)

A preparation step was necessary prior to introduction of the oil into the GC/MS for the individual determination of fatty acid composition. FAMEs were obtained by trans-esterification with sodium methylate in metanol. 0,5 ml of a 0,5% (w/v) solution of sodium methylate in metanol and 100 µl oil were mixed and shaken vigorously for 15 min in Bandelin ultrasonic shaker (Berlin, Germany). Oil was methylated 12 h (room temperature), and then 1 ml hexane was added to collect the FAME in hexane as above and analysed by GC/MS.

2.5. Sample Digestion

An Ethus Plus microwave labstation (Milestone, CT, USA), with computer-controlled easywave software was used to digest oil and pulp samples. 1 g of the oil sample and 1 g of the pulp sample were digested. The samples were digested using a mixture of nitric acid (4 ml) and hydrogen peroxide (2 ml) in a microwave digestion system. The samples were digested according to the following program (power [W]/time [min]): 20/2, 0/1, 250/2, 600/1, 400/5, ventilation time 3.0 min and measured by ICP/OES.

2.6. Analysis of FAME by GC/MS

A GC/MS (Shimadzu 17A-GC/MS QP5050, (Kyoto, Japon) and an auto sampler (Shimadzu AOC 20i, Kyoto, Japon). A 50 m * 0.32 mm ID * 0.32 µm film CP-WAX fused capillary column (Varian Inc., Lake Forest, USA), and GCMS Real Time Analysis software system (Shimadzu, Kyoto, Japon) were used for analysing FAME. The split ratio was 1:20 and the flow-rate of carrier gas (helium) 2ml min^{-1}. The injector and detector temperatures were fixed at 250°C. The temperature programme for the column was: hold ar 60°C for 1 min increase by 13°C/min to 175°C, increase at 4°C/min to 215°C, and then hold at 215°C for 35 min., total run was 86 min.

The mass spectrometer was operated in EI mode at 70 eV scanning the range 30-500 m/z in a 1 s cycle, in a full scan acquisition mode. All mass spectra were also compared with the data system library (Wiley 275).

2.7. Analysis of FAME by FT-IR

All infrared spectra were acquired using a Perkin Elmer BX FTIR spectrometer (Perkin-Elmer Norwalk, CT, USA) equipped with a mercury cad detector and KBr optics. Measurements were obtained at 4 cm^{-1} between 4000-600 cm^{-1}. For oil sample was placed on the ZnSe single bounce attenuated total reflectance (ATR) accessory. For pulp, KBr pellet technique was used. The spectra were accumulated from 32 scans. The spectrometer was connected to a computer using Perkin-Elmer Spectrum Windows software to manipulate the spectra.

2.8. Analysis of C, H, and N by Elemental Analyzer

C, H, and N content was determined by using a LECO CHNS-932 analyzer (St. Joseph, MI).

2.9. Analysis of Trace Elements by ICP/OES

The atomic absorption were performed using a Perkin Elmer PE 5300 DV ICP/OES spectrometer. The instrument-conditions parameters and limits of detection for the ICP/OES were summarized in Table 1. Standart stock solutions (1g/l) were used.

Table 1. Selection of lines and limits of detection (LOD) for the ICP/OES of elements in olive oil and pulp

Element	Wavelength/nm	LOD (mg/l)
Na	589,6	0,08
K	766,5	0,28
Mg	285,2	0,006
Ca	317,9	0,005
Mn	257,6	0,001
Fe	238,2	0,002
Zn	206,2	0,002
Cu	327,4	0,003

3. Results and Discussion

3.1. Fatty Acid Composition

The results obtained after 8h Soxhlet extraction and derivatization are given in Table 2. A total ion chromatogram of the GC/MS analysis of grape seeds oil are shown in Fig. 1.

Table 2. Percentages of FAMEs on the amount of sample and retention times

Sample (R_T)	C16:0 (Palmitic) (48,2)	C16:1 (Palmitoleic) (49,0)	C18:0 (Stearic) (58,9)	C18:1 (Oleic) (59,9)	C18:2 (Linoleic) (62,0)	C18:3 (Linolenic) (64,9)
Ratio, %	10,57	0,74	6,13	29,10	52,26	0,75

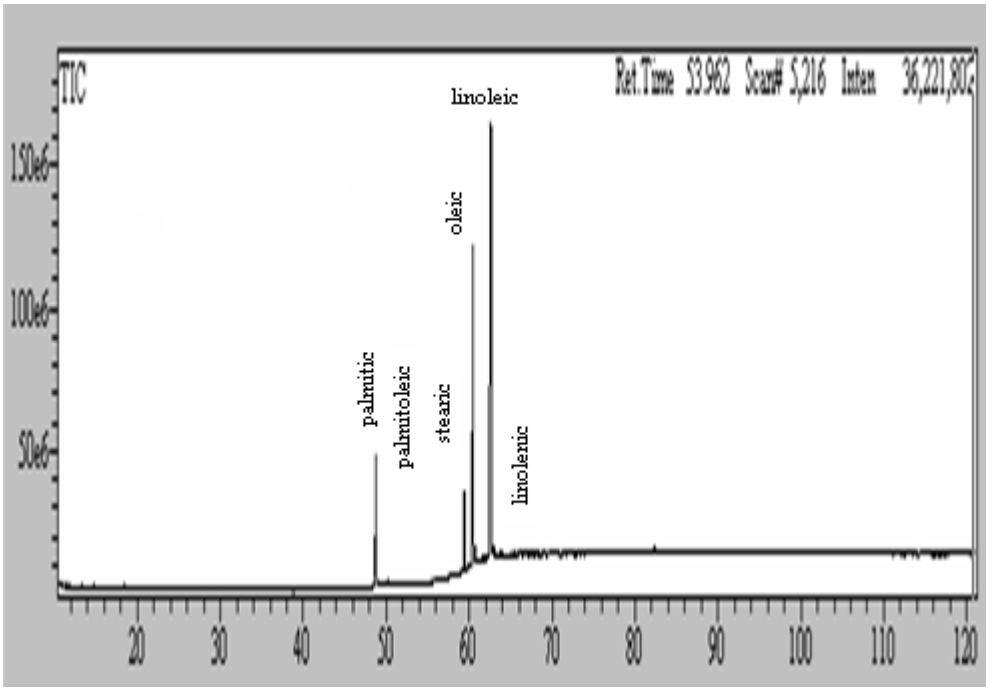

Fig. 1. Chromatogram obtained for the grape seed oil sample

Hexane was selected as our working. Because hexane produced higher atom efficacy than other solvents. Also it is suitable to the oil extraction and cheap [4, 25, 26, 27, 28, 29, 30, 31].

Saturated fatty acid values were less than the values of monosaturated fatty acids and also polyunsaturated fatty acids in grape seed oil. The ratio of MUFA, PUFA and SFA of grape seed oil were 29,84%, 53,01% and 16,7% respectively. Two types of PUFA n-6 (linoleic) and n-3 (linolenic), are important with regard to health, disease and stability of grape seeds [2]. The ratios of PUFA/SFA was 3,17. In addition, this the predominating linoleic acid, oleic acid showed an amount of 52,26% and 29,1%. In the chemical composition of grape seed oil the following fatty acids are found: palmitic acid 7,2-8,5%, stearic acid 3,8-3,9%, oleic acid 15,4-15,6%, linoleic acid 71,7-73,1%, linolenic acid 0,3-0,6% [4]. In our work, among the identified fatty acids, linoleic acid (C18:2) was prodominant. The ratio of linoleic acid was 52,26%. Second active component was oleic acid (C18:1) and showed an amount of 29,10%. These values are in general, in agreement to those determined in grape seed oil by Lague-Rodríguez et al. and Tangolar et al. [4,5].

3.2. FTIR Spectral Analysis

The representative spectra of grape seed oil and pulp are shown in Fig. 2. The oil spectra showed a typical characteritic of absorption bands for common triglyceride and also fatty acid. The assigment of prominent peaks were given in Table 3.

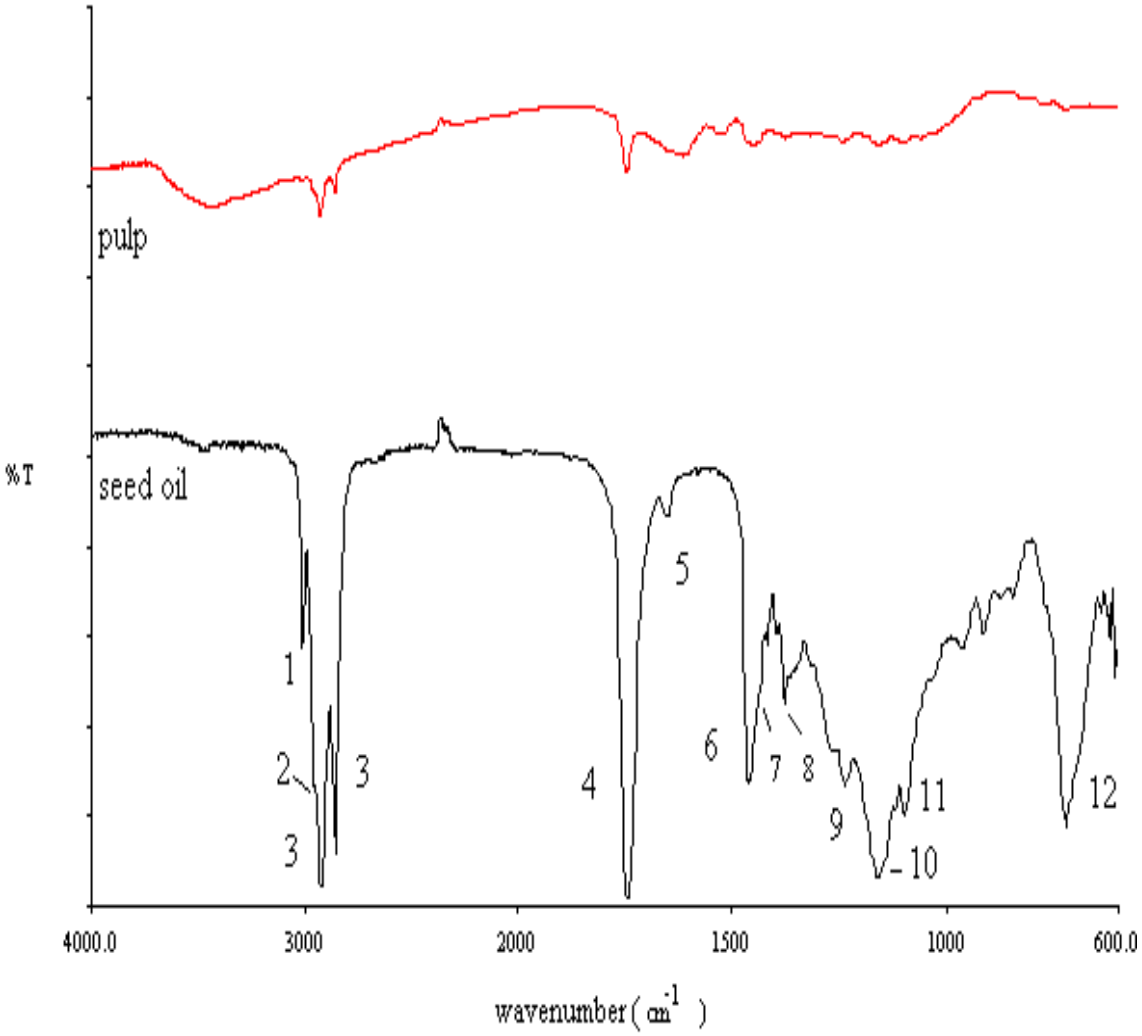

Fig. 2. The typical spectra of grape seed oil and pulp at frequency 4000-650/cm

Table 3. Functional groups and modes of vibration in grape seed oil and pulp*

Assigment	Frequency (/cm)	Functional grup vibration
1	3,009	C-H streching vibration of the cis-double bond (=CH)
2	2,954	Asymetric streching vibration of methyl (-CH_3) group
3	2,924 and 2,852	Asymetric and symetric streching vibration of methylene (-CH_2) band
4	1,743	Carbonyl (C=O) functional group
5	1,654	Cis C=C
6	1,465	Bending vibration of CH_3 and CH_2 aliphatic groups
7	1,417	Rocking vibration of CH bond of cis-disubstitued alkenes
8	1,377	Symetric bending vibration of CH_3
9-10	1,228 and 1,155	Vibration of streching mode from C-O group in esters
11	1,111	-CH bending and –CH deformation vibration of fatty acids
12	721	Overlapping of methylene (-CH_2) rocking vibration and to the out of plane vibration of cis- disubstitued olefins

* 32,33

Grape seed oil contains more unsaturated fatty acids (Table 2), especially oleic and linoleic acid, as determined using GC/MS. The presence of unsaturated fatty acids in seed oil can be observed in its specturum at 3,009/cm.

3.3. Elemental Analysis

Elemental analysis results are listed in Table 4.

Table 4. Results of elemental analysis of grape seed

Sample	C%	H%	N%	S%
1	51,55	6,01	1,47	-

-; not detected

In the C, H and N composition of grape seed oil are found: C 50,14%, H 7,07%, N 1,94% [30]. Grape seed is the only raw material with a significant nitrogen content, which is showed in the thermal behavior under both ambient and carbon dioxide atmospheres [6]. These values are in general, in agreement to those determined in grape seed by Lupascu1 et al. [6].

Table 5 shows the results for the determination of the elements in grape seed oil and pulp by ICP/OES. The detection power of ICP was enough for the determination of Na, Mg, Ca, Mn, Fe, Zn and Cu. Pulp sample contain higher amount of K (3,820 mg/g) and Ca (5,290 mg/g).

Table 5. Results for the determination of elements in grape seed pulp and oil by ICP/OES

Elements	Pulp (mg/g)	Oil (mg/g)
Na	0.072 ± 0.003	0.114 ± 0.002
K	3.820 ± 0.050	0.006 ± 0.004
Mg	0.985 ± 0.010	0.062 ± 0.001
Ca	5.290 ± 0.080	0.427 ± 0.015
Mn	0.039 ± 0.001	0.030 ± 0.001
Fe	0.024 ± 0.010	0.038 ± 0.001
Zn	0.012 ± 0.001	0.006 ± 0.001
Cu	0.008 ± 0.001	0.001 ± 0.000

The measurements were performed using six calibration solutions for the external standardization and analyte additions. Straight lines made a good approximation of all calibration curves obtained (R: 0.999). Low limits of detection obtained for real oil and pulp samples using a simple analytical procedure, high repeatability and long-term stability of analytical signals allow admonition of the method for routine analysis. The results of mineral analysis showed that samples contained amount of different elements.

4. Conclusion

Grape seeds could be used as a food supplement and wine industries. The grape seeds were evaluated in terms of quality properties including fatty acid composition, element

and mineral contents. In this study ratio analysis of fatty acid methyl esters was performed. The presence of FAMES composition in grape seed oil can be detected through FTIR spectroscopy using the ATR sampling method. FTIR spectroscopy can be used to monitor the adulteration of oil.

The determination of trace elements, C, H and N in oil and pulp samples by using ICP/OES and elemental analyzer. ICP/OES after microwave-assisted digestion represents a reliable and simple analytical method for oil analysis.

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