RESEARCH OF THE CHEMICAL COMPOSITION OF GRAPE OIL OBTAINED FROM THE EXTRACTION PROCESS WITH THE PARTICIPATION OF VARIOUS SOLVENTS

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Abstract: *Efficient processing of rapidly increasing plant waste, environmental safety, and sustainable use of resources have become important areas in the chemical industry in modern times. Grape seeds are rich in highly unsaturated fatty acids and polyphenol compounds and are selected as a promising raw material for the production of environmentally friendly, plant-based grape seed oils due to their high production as a by-product in the grape processing industry. The article studies the process of extracting oil from the seeds of "Madrasa" and "Bayan-Shira" grape varieties, which are waste, with the participation of solvents (hexane, acetone). The effect of the polarity difference of grape varieties and solvents on the yield and biochemical composition of grape seed oil was studied. 15.4% oil was obtained from the extraction of the seeds of the "Madrasa" grape variety with hexane, and 7.8% from the extraction of the seeds of the "Bayan-Shira" grape variety with acetone. Polyphenols and saturated fatty acids in the grape seed oil fraction were determined by gas chromatography. The presence of components such as chromophore groups, phenols, and tocopherols in the grape seed oil was analyzed by ultraviolet (UV) spectroscopy.*
Keywords*: grape, hexane, acetone, extraction, Linoleic fatty acid, Oleic fatty acid*

1. Introduction

The evaluation of plant-derived biomaterials in terms of functional components is one of the main directions of research in the field of modern chemistry and green technologies [1]. In recent decades, the volume of research conducted on the evaluation of plant-derived wastes, agricultural by-products, household and agro-industrial wastes, technical plants, and the development of technologies for their processing into high-value-added biological and environmentally friendly chemical products has increased. One of the most commonly used plant-based raw materials in this field is grape seeds, which are a major by-product of the viticulture industry [2]. As a result of the dynamic growth of grape production worldwide, the amount of plant-derived wastes such as seeds and skins has also increased, thus, the use of these raw materials as valuable components has become strategically important in terms of both reducing the environmental load and increasing economic efficiency [3]. The richness of grape seed oil in bioactive substances, its high nutritional value, and its wide application possibilities have led to the development of this raw material as a potential resource in the food industry, as well as in areas such as cosmetics, pharmacy, and bioenergy. At the same time, the results obtained are of scientific and practical importance in terms of converting plant waste from various fields into valuable resources and are fully consistent with the principles of modern green chemistry [4].

The composition of grape seed oil is quite complex from a phytochemical point of view and is characterised by the richness of various biologically active components. Although this oil is formed on the basis of a lipid matrix dominated by triglycerides, the main substances that determine its functional value are components of an antioxidant nature, such as polyphenols, flavonoids, tocopherols, sterols and carotenoids [5]. This bioactive complex composition of the oil significantly determines its resistance to oxidation, the spectrum of biological effects and the possibilities of technological applications. That is why the determination of the phytochemical and lipid profile of

grape seed oil by analytical methods has been the focus of attention of scientific laboratories in recent years [6, 7].

One of the main factors affecting oil yield and chemical composition is the nature of the solvent used in the extraction process [8]. The types of solvent and the difference in polarity, selectivity, and diffusion properties directly affect the extraction of both triglycerides and polar bioactive substances such as polyphenols and tocopherols from the oil. Non-polar solvents such as hexane demonstrate high efficiency for the extraction of mainly neutral lipids and are widely used in industry [9]. In contrast, polar solvents such as ethanol and acetone have a higher extraction capacity for phenolic compounds and other polar components. This difference affects both the quantitative and qualitative parameters of the extract and determines the oxidative stability, biological activity and technological value of the oil. Therefore, comparative extraction with different solvents and the study of their effects on the compositional and spectral properties of the oil are among the priority directions in modern lipid chemistry. This approach allows for both a comparative scientific evaluation of various extraction methods and the identification of technological optimization opportunities for the use of grape seed oil in areas such as food, biotechnology, and bioenergy [10].

Polyphenols are one of the most important antioxidant components in plant-based raw materials and play a special role in assessing the quality indicators of oil. Among grape seed polyphenols, catechin and epicatechin derivatives, proanthocyanidins, gallic acid, caffeic acid, flavonoids, and other aromatic compounds occupy an important place [11]. The amount and component composition of polyphenols vary depending on the genetic characteristics of the grape variety, cultivation conditions, seed maturity and extraction technology. A high content of these substances reduces the oxidation rate of the oil, increases its storage life and plays an important role in maintaining oxidation stability in technological processes, especially in biofuel production [12]. In this regard, the analysis of polyphenols by various spectroscopic methods (ultraviolet and infrared spectroscopic) is considered a fundamental analytical analysis for assessing the chemical and biofunctional value of the oil. The combined application of analytical methods allows for a more accurate assessment of the quality parameters of grape seed oil and a more in-depth study of the mechanisms by which the choice of solvent affects the physicochemical properties of the oil [13].

Unsaturated fatty acids are one of the most important lipid components of vegetable oils, and the presence of one or more double carbon-carbon (C=C) bonds in their chemical structure sharply distinguishes this class from saturated fatty acids functionally and chemically. The fatty acid composition of grape seed oil is characterized by a high content of unsaturated fatty acids; in particular, linoleic (C18:2) acid constitutes the majority of the fatty acids. Oleic (C18:1), palmitic (C16:0) and stearic (C18:0) acids, although present in relatively small amounts, affect the physicochemical properties of the oil. This combination of fatty acids forms important properties of the oil, affecting its oxidative stability, viscosity, freezing point, cold flowability and potential for use as a biofuel [14].

The analysis of the literature data [15, 16] showed that although the composition of grape seed oil consisting of chemical and biologically active components has been investigated in various studies, the importance of systematic studies that simultaneously evaluate the complex effect of extraction using different solvents on the polyphenol content, fatty acid composition and spectral properties should be studied. Taking this into account, the *aim of the study* is to comparatively investigate the process of extracting oil from the seed waste of different grape varieties ("Madrasa", "Bayan-Shira") in the presence of solvents (hexane, acetone), the polyphenol and fatty acid composition of the oil, and various physicochemical properties. At the same time, in this study, the effect of grape seed oil extraction using solvents with different polarity differences – hexane and acetone – on the polyphenol content of the oil, the fatty acid profile determined by gas chromatography, and the spectral properties analyzed by UV (ultraviolet) spectroscopy was studied in a comprehensive manner.

2. Experimental part

The seeds of two grape varieties, “Madrasa” and “Bayan-Shira”, were used as raw materials. In order to separate the oil from the seeds of the grape varieties by extraction method, the seeds were initially prepared. The seeds were dried at room temperature at 23°C until the final moisture content reached 5%. The dried seeds were ground into powder using a Retsch MM400 laboratory mill with a frequency of 30 Hz and passed through a standard sieve of 40 mesh size. Hexane was used as a non-polar solvent, and acetone was used as a polar solvent to separate non-polar lipids (mainly triglycerides and free fatty acids). The hexane and acetone solvents used were purchased from Sigma Aldrich (St. Louis, MO). The crushed grape seeds and solvents were taken in a ratio of 1:10 (seed:hexane 1:10; seed:acetone 1:10) and mixed continuously for 2 hours at room temperature in a device consisting of a magnetic stirrer. The maximum separation of the solvent from the obtained grape seeds and hexane and grape seeds and acetone mixtures was carried out by filtration in a Büchner funnel using Whatman No. 2 filter paper under low vacuum. The oil in the upper layer of the extract was collected and passed over a 2.5 cm thick layer of anhydrous sodium sulphate. Hexane and acetone were separated under vacuum at 40°C using an R-200 rotary evaporator (Büchi Corporation, Switzerland) and were reused as solvents in subsequent experiments.

Polyphenols in grape seed oil were separated using methanol. A mixture was prepared by adding 2 ml of methanol to 0.5 ml of grape seed oil. The mixture was centrifuged at 10000 rpm for 10 min. The supernatant was collected using a glass transfer pipette and analyzed for total polyphenols using the Folin-Ciocalteu reagent method [17]. Gallic acid equivalents (μg GAE) were used for the starting oil.

The extraction process and polyphenol separation experiments carried out to obtain the oil were repeated three times.

The saturated fatty acids in the separated grape seed oil fraction were determined by gas chromatography on an AutoSystem XL (Perkin Elmer, Canada) chromatograph with a flame ionization detector. A 100 m long thin quartz capillary column (diameter 250 μm x 0.53 mm ID x 0.50 μm layer thickness) was evaporated at 250°C. A 1 μl sample volume was used in the column. Both the injector and detector temperatures were 250°C. Under the influence of a carrier gas (helium, 7 ml/min, constant flow) constantly flowing through this tube, the grape seed oil in vapor form moved through the tubes. At the same time, the oven temperature of the column started at 50°C and increased to 250°C at a rate of 3-4°C/min and was maintained at 250°C for 25 minutes. The analysis and concentration of free fatty acid components in grape seed oil were calculated according to the chromatogram obtained using a GC-463 standard mixture (Nu Check, Elysian, MN, USA) under gas chromatography conditions.

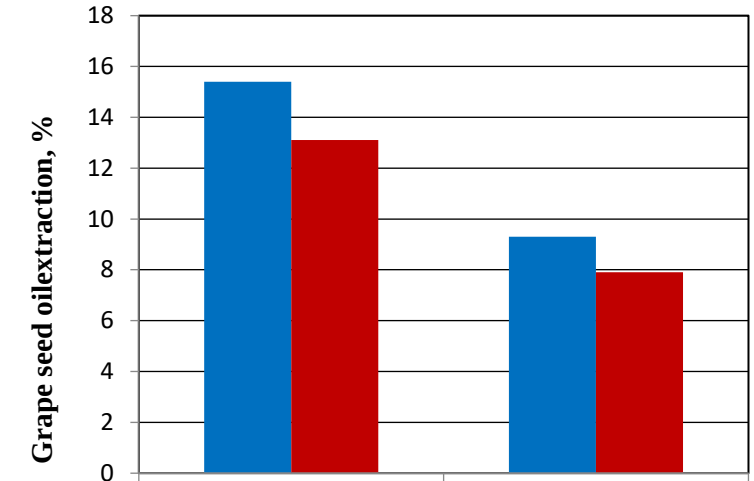
Grape seed oil yield and total hydrocarbon content data were determined as the mean ± standard deviation.

The presence and nature of bonds in the structure of fatty acids, which are the basis of grapeseed oil, were determined by the ultraviolet (UV) spectroscopic method. UV spectroscopic analyses of the samples were carried out with a Thermo Scientific Evolution 300 model UV-Vis spectrophotometer. Measurements were carried out in the wavelength range of 180–900 nm, at room temperature (≈25°C). For analysis, oil samples were first diluted with hexane as a solvent in a ratio of 1:10 (v/v). The reason for using hexane as a solvent is that it does not absorb UV radiation after 200 nm, and the spectra of the oil components are clearly observed. After preparing a solution from the mixture in a homogeneous phase, it was used for measurement.

3. Results and discussion

According to the analysis results of the extraction experiments of the seeds of the “Madrasa” and “Bayan-Shira” grape varieties with the presence of hexane and acetone as solvents, the effect of the solvent and grape varieties on the oil yield obtained was determined and shown in Fig. 1. The lowest oil yield (7.8%) was obtained from the seeds of the “Bayan-Shira” grape, and the highest oil yield (15.4%) was obtained from the seeds of the “Madrasa” grape, depending on the extraction solvent. In the extraction process carried out with the participation of the same solvent, the high oil

yield in the seeds of the "Bayan-Shira" and "Madrasa" grape varieties was obtained from the extraction process carried out with the participation of the same solvent, and in the extraction process carried out with the participation of hexane and acetone solvents of the same grape varieties, the high oil yield was obtained from the extraction process carried out with the hexane solvent.



	Madrasa	Bayan-Shira
Hexane	15,4	9,3
Acetone	13,1	7,9

Fig. 1. Amount of grape seed oil obtained from different grape varieties in the presence of extraction solvents

The results of the study showed that the differences in oil yield between grape varieties, in addition to the complex effect of genetic, morphological and biochemical characteristics, also differ between varieties in the degree of unsaturation of fatty acids. In varieties with high unsaturated fatty acids, since the solubility of oils in solvents is higher, an increase in the yield of the obtained oil is observed. At the same time, in varieties with high polyphenol content, the strength of the cell wall may complicate extraction, which leads to a decrease in oil yield [18].

The main reason why the oil yield obtained from the extraction process using hexane as a solvent is higher than the oil yield obtained from the extraction process using acetone is related to the degree of polarity, lipophilicity, and solubility of the solvents used. The high oil yield obtained from the seeds of "Madrasa" and "Bayan-Shira" grapes (15.4-9.3%) is due to the fact that hexane has a non-polar structure and is highly compatible with triglycerides, free fatty acids and other hydrophobic components that constitute the main components of the oils. The relatively low oil yield obtained from the seeds of "Madrasa" and "Bayan-Shira" grapes (13.1-7.9%) is due to the moderately polar structure of the acetone used as a solvent.

The total polyphenol content of grape seed oils was determined and presented in Fig. 2. As can be seen from the figure, the total polyphenol content of grape seed oils varies significantly depending on the grape varieties and extraction solvents. Regardless of which solvent is used, the total polyphenol content of grape seed oil obtained from the seeds of the "Madrasa" variety is higher. The total polyphenol content of the oil obtained from the extraction of "Madrasa" grape seeds with hexane (580 µg GAE/g oil) is relatively low, while the total polyphenol content of the oil obtained from the extraction with acetone (403 µg GAE/g oil) is relatively low. The total polyphenol content of the oil obtained from the extraction of "Bayan-Shira" grape seeds with hexane (530 µg GAE/g oil) is relatively low, while the total polyphenol content of the oil obtained from the extraction with acetone (380 µg GAE/g oil) is relatively low. The main polyphenols identified in grape seed oil are reported to be catechins, epicatechins, trans-resveratrol, and procyanidin B1 [19]. Although considered hydrophilic, these phenolic compounds have very low water solubility. The higher total polyphenol content in both grape seed oils extracted with hexane suggests that the hydrophobicity of the phenolic compounds is greater than their hydrophilicity.

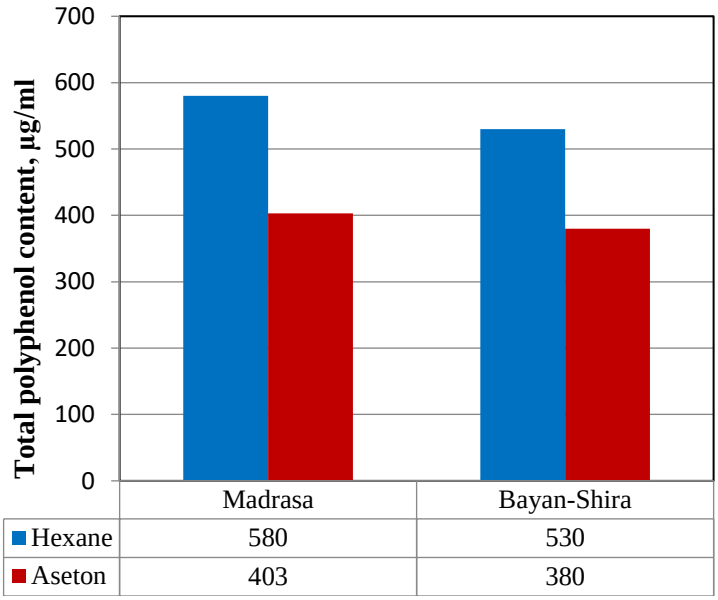


Fig. 2. Total polyphenol content of grape seed oil obtained from different grape varieties in the presence of extraction solvents

Fatty acids in grape seed oil obtained from the extraction of "Madrasa" and "Bayan-Shira" grape seeds with the participation of hexane and acetone solvents were determined by gas chromatography analysis. As a result of the analysis, the typical fatty acid composition, percentage and chemical formula of grape seed oil are given in Table 1. As can be seen from the table, the fatty acids of the obtained grape seed oil consist of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and gadoleic acid (C20:0). Between the two types of grape seed oil, the seeds of the "Bayan-Shira" variety have a lower percentage of fatty acid content than the "Madrasa" variety.

Table 1. Free fatty acid composition of grape seed oils obtained from hexane and acetone extraction of grape varieties

Fatty acids	Chemical formula	Grape seed oil obtained by extraction with the participation of hexane		Grape seed oil obtained by extraction with the presence of acetone	
		Madrasa	Bayan-Shira	Madrasa	Bayan-Shira
Myristic C14:0	$C_{14}H_{28}O_2$	0.47	0.38	0.74	0.56
Palmitic C16:0	$C_{16}H_{32}O_2$	6.31	5.52	6.04	5.78
Palmitoleic C16:1	$C_{16}H_{30}O_2$	0.1	-	0.1	-
Stearic C18:0	$C_{18}H_{36}O_2$	3.11	2.98	3.47	3.33
Oleic C18:1	$C_{18}H_{34}O_2$	13.05	12.12	15.19	15.17
Linoleic C18:2	$C_{18}H_{32}O_2$	69.03	68.28	67.18	64.59
Linolenic C18:3	$C_{18}H_{30}O_2$	6.89	6.48	6.24	6.89
Gadoleic C20:1	$C_{20}H_{40}O_2$	0.14	0.47	0.21	0.12
Behenic C22:0	$C_{22}H_{44}O_2$	0.2	0.1	0.24	1.11
Total:		99.30	96.33	99.41	97.55

The oil obtained from the hexane extraction of the seeds of the same grape variety has a higher content of fatty acids than the oil obtained from the acetone extraction. The presence of unsaturated fatty acids, especially linoleic acid (C18:2) and linolenic acid (C18:3) in grape seed oils will significantly reduce the oxidative stability of the oil [20]. The oleic fatty acid (C18:1) contained in the oils slightly increases the oxidative stability, which has a positive effect on the flowability and

viscosity of the oil. The low content of saturated fatty acids causes the oils to have a low melting point and maintain a liquid state. The content of long-chain saturated fatty acids gadoleic acid (C20:1) and behenic acid (C22:0) is very low (<1%), which indicates a weak tendency of the oil to crystallize.

The UV spectra of oil samples obtained from the extraction of the "Madrasa" grape variety with hexane and the extraction of the "Bayan-Shira" grape variety with acetone were recorded with a spectrophotometer in the range of 200–400 nm and were analyzed comparatively. The results of the UV spectroscopy analysis of oils obtained from grape seeds are given in Figure 3 and Tables 2 and 3. The results related to the absorption range at each wavelength are recorded in the presented UV spectrum and tables. As can be seen from the tables, the composition of grape seed oil consists of components consisting of several main functional groups. The results of spectral analyses indicate that the presence of multiple absorption maxima in the 200–300 nm interval for both samples indicates the presence of lipid oxidation components in the oil, including conjugated and non-conjugated dienes, tocopherols, phenolic antioxidants, and chromophore groups. This range evaluates the amount of bioactive components and oxidation degree in the oil, which are characteristic of vegetable oils [21].

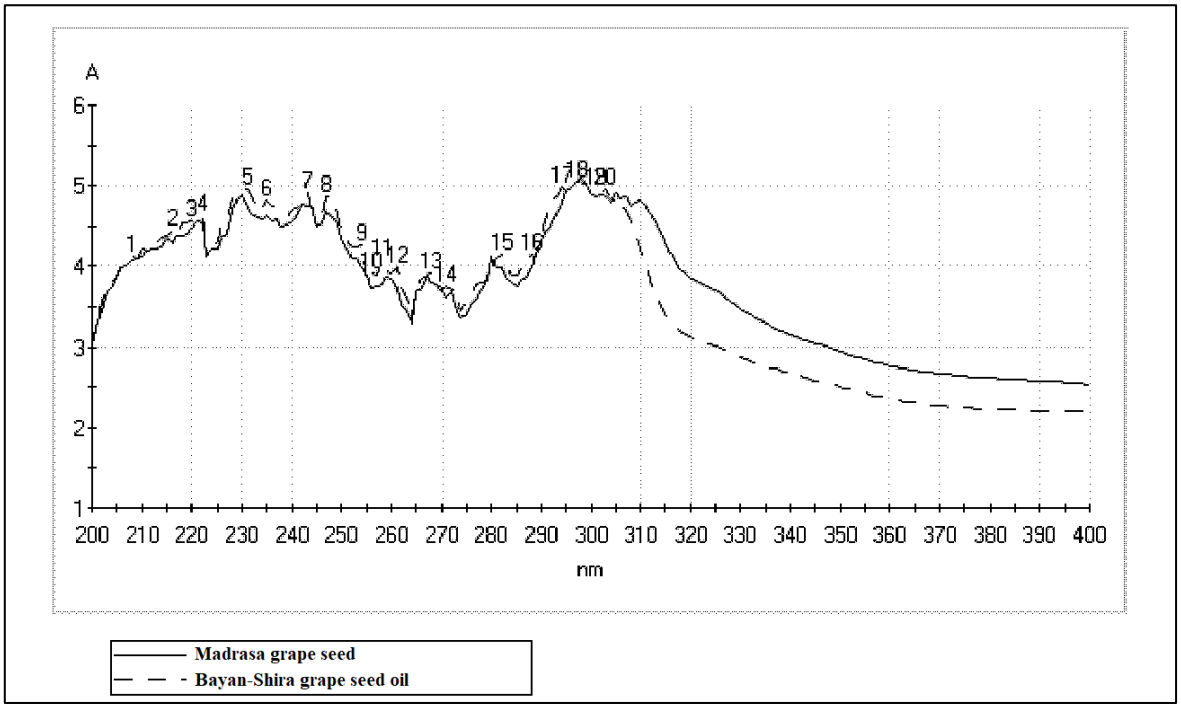


Fig. 3. UF spectrum of grape seed oil obtained from different grape varieties in the presence of extraction solvents

Table 2. UV-spectrum absorption bands λ_{max} (nm) and molar absorption coefficient (ε L. mol^{-1}. cm^{-1}) of active compounds contained in the oil obtained from the extraction of "Madrasa" grape seeds with hexane as a solvent

λ_{max} (nm)	Absorbans	ε L. mol^{-1}. sm^{-1}
215	4.356	43.56
221	4.570	45.70
230	4.893	48.93
235	4.640	46.40
237	4.579	45.79
242	4.784	47.84
247	4.685	46.85
259	3.863	38.63
267	3.879	38.79
272	3.689	36.89
280	4.115	41.15

298	5.097	50.97
302	4.888	48.88
305	4.907	49.07
307	4.872	48.72
310	4.816	48.16

Table 3. UV-spectrum absorption bands λ_{max} (nm) and molar absorption coefficient (ε L. mol^{-1}. cm^{-1}) of active compounds contained in the oil obtained from the extraction of "Bayan-Shira" grape seed with acetone as a solvent

λ_{max} (nm)	**Absorbans**	**ε L. mol^{-1}. sm^{-1}**
208	4.128	41.28
216	4.440	44.40
220	4.567	45.67
222	4.628	46.28
231	4.957	49.57
235	4.830	48.30
243	4.901	49.01
247	4.871	48.71
254	4.274	42.74
256	3.927	39.27
258	4.077	40.77
261	3.979	39.79
268	3.926	39.26
271	3.747	37.47
282	4.149	41.49
288	4.141	41.41
294	4.985	49.85
297	5.052	50.52
301	4.964	49.64
303	4.961	49.61

Although oils were obtained from different grape varieties in the presence of different solvents, a large number of peaks are observed in both oil samples between 200 and 310 nm. This reflects the polycomponent nature of grape seed oil and, in particular, the peaks belonging to p-hydroxybenzoic acid derivatives; catechin and epicatechin fractions; gallic acid tocopherol and tocotriferols; and conjugated lipid groups known in these oils [22]. The large number of peaks and the differences in intensity reflect the differences in the chemical composition of both varieties. The peaks observed in the spectrum are mainly characteristic of unconjugated dienes and some simple phenolic acids in the 220–240 nm range; tocopherols (vitamin E fractions) and aromatic phenolic compounds in the 250–280 nm range. The decrease in intensity in the zone after 300 nm in the 300–400 nm range indicates that the electronic transitions of chromophore groups in the oil are limited. This confirms that grape seed oil is pure and unoxidized. In oxidized oils, special absorption peaks (extinction) are observed in this area, but according to the results of the analyses, this effect is not observed in the oils obtained. The higher absorption intensity in the oil sample of the "Madrasa" grape variety indicates that the oil is richer in lipid groups, polyphenols or phenolic substances, antioxidants and tocopherols. Although the intensity of the peaks in the "Bayan-Shira" variety is relatively low, the spectral profile is the same, that is, the types of components are the same, only the quantity is different.

Analysis of the obtained results shows that in the oil sample of the "Madrasa" grape variety, the absorption spectrum at λ_{max} 221 nm is 4.570 A and the molar absorption coefficient ε. 45.70; the absorption spectrum at λmax 298 nm is 5.097 A and the molar absorption coefficient ε. 50.97; in the oil sample of the "Bayan-Shira" grape variety, the absorption spectrum at λ_{max} 222 nm is 4.628 A and the molar absorption coefficient ε. 46.28 belongs to the π-π* transitions.

Conclusion

As a conclusion of the conducted research, we can note that the yield of grape seed oil obtained varies depending on the grape variety and the solvent used in the extraction process. The extraction of grape seeds of the “Madrasa” variety yielded 15.4% oil using hexane and 13.1% using acetone, while the extraction of grape seeds of the “Bayan-Shira” variety yielded 9.3% oil with hexane and 7.8% with acetone.

The high content of polyphenol compounds in the obtained grape seed oil has a significant impact on its oxidation stability. Since polyphenols have strong antioxidant properties, it is recommended to use grape seed oil as a raw material for the production of environmentally friendly biofuel components. This property reduces the oxidation processes that occur when biofuels come into contact with oxygen, heat and light to a certain extent. This is especially a key factor in blends where these biofuel components are added to petroleum-based diesel fuel in various proportions, since the overall oxidation stability of the blend is determined by the stability of the biofuel. The high polyphenol content of grape oil obtained from the “Madrasa” variety increases the oxidation induction period of blends prepared from it as fuel, as a result, it prevents the formation of fuel deposits, tarring and an increase in the acid number.

The fatty acid composition of grapeseed oil varies depending on the grape variety and the solvent used in the extraction process. Gas chromatography analysis has determined that the main components of grape seed oil are linoleic (C18:2), oleic (C18:1), palmitic (C16:0) and stearic (C18:0) fatty acids. Oils obtained from the extraction process using hexane as the solvent have been found to contain higher amounts of C16:0, C18:0, C18:2 fatty acids, but lower amounts of C18:1 fatty acids, than oils obtained from the extraction process using acetone. Grape seed oil obtained from the extraction of “Madrasa” grape seeds with hexane has a higher overall content of unsaturated fatty acids. The results showed that due to the difference in polarity of the solvents used in the extraction process from the seeds of different grape varieties, the amount of fatty acids in the oil obtained also differed to a certain extent. At the same time, since grape seed oil contains a high amount of unsaturated fatty acids, it can be used as a raw material in the preparation of biofuels. Because methyl esters (biodiesel) obtained on the basis of unsaturated fatty acids have a high oxygen content, they increase the combustion efficiency in the engine, reduce harmful gas emissions (CO, HC) and keep the calorific value of the fuel constant. Also, since unsaturated fatty acids have double bonds in the carbon chain, they reduce the viscosity of the oil, increase its cold flow properties and facilitate the homogeneous mixing of biodiesel with petroleum-based diesel.

It is recommended to use grape seed oil obtained from the varieties "Madrasa" and "Bayan-Shira" with the participation of various solvents as the most promising raw material for the preparation of biofuel components. The main reason for adding these components to petroleum-based diesel fuel is to increase the physicochemical parameters of the fuel and reduce the ecological problems it causes in the environment.

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