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SEED OIL CONTENT AND SELECTED QUALITATIVE PARAMETERS OF OILS FROM GRAPE SEEDS

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ABSTRACT

Grape seed oil (*Oleum vitis viniferae)* represents promising plant oil, which is used mainly in gastronomy and for pharmaceutical purposes as well as for various technical applications. In this paper, there were examined oil contents and oil quality properties of seeds taken from 8 grape cultivars. Oil contents were found to be different for each cultivar, which ranged from 11.5% (Dornfelder) to 17.5% (Riesling). The results showed a dependence between the length of the growing season for individual varie-ties and the total content of oil in seeds. Fatty acid concentrations in the evaluated oil samples were in various ranges, while the highest values were determined in linoleic acid 70.10 to 71.55%, oleic acid 15.61 to 17.14%, palmitic acid 6.87 to 8.18% and stearic acid 3.16 to 3.90%. Saturated fatty acid values were lower than the values of monounsaturated fatty acids and polyunsaturated fatty acids in all oil samples. The degree of unsaturation in the grape seed oil ranged between 88.6 – 89.21%. Thanks to its content, grape seed oil can be considered as a food supplement improving the nutri-tional value of the human diet.

Keywords: grape cultivars; grape seed oil (*Oleum vitis viniferae*); fatty acid composition; cold screw pressing

INTRODUCTION

The grape wine is the most commonly grown kind of fruit around the world. Currently the area of vineyards is about 8 million hectares, where Europe occupies approximately 57% of this hectarage, i.e. about 4.5 million hectares. According to the information from Organisation Internetionale de la Vignet et du Vin **(OIV, 2009)**, 66.5 million tonnes of grapevine are processed every year. From this amount, 38 million tonnes of grapevine are processed in Europe. Production of grapes is generally situated in moderate-warm climate zones, e.g. Italy (9.3 mt.year^{-1}), France (6.8 mt.year^{-1}), USA (6.4 mt.year^{-1}), Spain (5.9 mt.year^{-1}) but also China (5.7 mt.year^{-1}) according to the information from 2006 **(FAO, 2006)**.

Grape pomace, the residue of wine processing, accounts for 20% of grape (v/w). Winemaking wastes, traditionally considered as an economic and environmental problem, are now becoming increasingly recognised as valuable commodities for the production of value added products, such as grappa or vine seed oil **(Passos et al., 2009)**. Pomace consists by 20 – 26% of grape seeds, 7.8 – 11% of protein and 10 – 20% of fatty oil depending on pressing conditions **(Bockisch, 1993; Schieber et al., 2002)**.

On average, grape seed oil is by 90% composed of poly- and monounsaturated fatty acids, which are responsible for its value as nutritive edible oil, particularly of linoleic acid (58 – 78%, 18:2n-6) followed by oleic acid (3 – 15%, 18:1n-9) and minor amounts of saturated fatty acids (10%). Unrefined oils contain bioactive compounds including tocopherols (5 – 52 mg.100 g^{-1}) and numerous phenolic components, consisting of low and high molecular plant phenolics, which may contribute to beneficial effects of vegetable oils **(Bockisch, 1993; Firestone, 1999; Morin, 1996; Frančáková et al., 2015)**.

The aim of the study was to compare seeds of 8 varieties of vine and their evaluation from the perspective of oil content and oil quality properties such as fatty acid composition.

Scientific hypothesis

Different grape varieties can affect contrast in oil content and its qualitative composition when pressed from seeds.

MATERIAL AND METHODOLOGY

Grape seeds

Collection of grape marc for the separation of seeds was carried out in the processing season 2016 at the Agropol Mikulov Company. A prototype of vibratory separator was used to separate the seeds from marc. This machine applies the principle of mechanical vibrations transmitted on three flat screens with different shapes and sizes of holes. Separation of seeds was carried out separately from marc from four white (Riesling, Pinot Gris, Pálava, and

Hibernal) as well as four red (Dornfelder, Blaufränkisch, Zweigelt and Laurot) must varieties of grapevine. For successful pressing of seeds and their storage, their initial moisture content was lowered from 43 – 49% to about 5 – 10% in a chamber dryer. The temperature in the chamber dryer did not exceeded 40 °C. Material was kept in a closed bag, at room temperature until screw pressing.

Screw pressing process

Before screw pressing experiments, the press head was pre-heated at the desired temperature for 20 minutes using a temperature-regulated heating ring. Pressing experiments were conducted without external heating (cold pressing). During pressing, grape seeds were fed into the press on demand by gravity through the hopper and the seed level was maintained constant to ensure constant press performance. Seeds of all varieties were pressed at the same speed of 40 rpm.

Analytical procedure – chemicals

There were used Sodium methoxide (Fluca), Boron trifluoride (Sigma Aldrich), 2,2,4-Trimethylpentane = isooctane (Sigma Aldrich), Nitrogen (6.0), Hydrogen (6.0) and technical air (Siad). The standard for identification: PUFA No. 3, from Menhaden Oil (Supelco) and IS (inner standard) for quantification: Methyl pentadecanoate (was purchased from Fluca) were used in analyses of fatty acid content.

Determination of water content and density

Water content of grape seeds was determined by dehydration at 103 °C according to CSN EN ISO 665 (461025) Oilseeds – Determination of moisture and volatile matter content. The analysis was made on 5 g of grinded sample, weighted with an accuracy of 0.1 mg. Results are expressed as the ratio of water loss per gram of dried sample. Determination of water content was performed in triplicate. Density of oil was determined pycnometrically according to CSN EN ISO 6883. This international standard specifies a method for the determination of the conventional mass per volume ("litre weight in air") of vegetable fats and oils.

Determination of the total lipid content in the seeds through extraction

To determine the total lipid content, the Soxhlet extractor (Kavalierglass, Czech Republic) was used with hexane as a solvent. Crushing the seeds of a given variety always took place immediately prior to the extraction of oil using a coffee blender (Moulinex, France), for 2 min. The emphasis was always placed on precise cleaning of the grinder in order to avoid distorting of results. For seeds of each variety after grinding, the water content of the sample was determinet. The temperature of the extraction mixture was kept by the heating mantle closely around the boiling point of hexane (70 °C). Extraction was always carried out for 32 hours. Subsequently, the hexane was evaporated and the sample weighed twice in two day intervals. During this time, the sample of oil was kept in a dark environment. Determination of oil content was performed in triplicate.

Analysis of fatty acids

In the analysis of fatty acids by gas chromatography, there is firstly necessary their transesterification. The extracted fat is after adding of 2 mL of isooctane with 10 mg of methyl pentadecanoate (inner standard) dissolved in ultrasound for 2 minutes. The mixture is afterwards heated with 2 mL under a reflux condenser. Subsequently, there are added 2 mL of boron fluoride to neutralize the unreacted sodium methanolate and in the acidic environment there are also esterified possible free fatty acids.

For the analysis of fatty acids, there was used a gas chromatograph HP 4890D (Hewlett Packard) with a flame ionization detector (GC-FID). The separation was performed on column DB-23 (60 m x 0,25 mm x 0.25 µm). For measuring, there was chosen the temperature program T1 = 100 °C, t1 = 3 min, 10 °C.min^{-1} on T2 = 170 °C, t2 = 0 min, 4 °C.min^{-1} on T3 = 230 °C, t3 = 8 min, 5 °C.min^{-1} on T4 = 250 °C, t3 = 15 min. The injector temperature 270 °C, detector temperature 280 °C, injection 2 µl. The flow divider was set up to the ratio 40:1. The nitrogen carrier gas flow was 1 mL.min^{-1}. The resulting chromatograms were processed using the station CSW (version 1.7, Data Apex, Praha). The samples were performed in triplicates.

Statisic analysis

Results were reported as means and standard deviation. Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests were conducted to determine the differences among means, (Statistica CZ, ver. 10). The statistical significance was declared at $p \leq 0.05$.

RESULTS AND DISCUSSION

The oil contents and selected oil quality properties of grape seeds obtained from 8 grape cultivars are shown in Table 1 and Table 2.

Oil Content

The results of values stated in Table 1 show that oil content of seeds of evaluated varieties moved from 11.5% for the variety Dornfelder to 17.5% for the variety Riesling (v/w). The experimentally determined values of oil content in grape seeds in this work, thus, correspond with literature data. For example, from 12.4% to 16.0% of oil in seeds of varieties typical for Turkey **(Baydar et al., 2007)**, or state the oil content in the range of 8 – 20% (dry basis) **(Crews et al., 2006)** and also states the range 7 – 20% **(Matthaus 2006)**. The experimentally determined level of oil 15.6 ±0.14% in the variety Zweigelt grown in the Czech Republic, corresponded very well with the value found in the same variety grown in Japan (15.4%) **(Ohnishi et al., 1990)**. This fact leads to the assumption, that a variety has a significantly higher influence on oil content in seeds than an actual location. The highest oil content was found in the variety Riesling and Laurot, which represent late-ripening varieties. The lowest oil content was determined in the variety Dornfelder, which belongs to middle early varieties. Thus, these results suggest a link between oil content and maturity of seeds.

Table 1 Oil content and selected qualitative parameters of oil from seeds of evaluated varieties.

Cultivar		Pinot Gris	Riesling	Hibernal	Pálava	Dornfelder	Blau-fränkisch	Laurot	Zweigelt
Oil content (%) (v/w)		16.3 ±0.08	17.5 ±0.15	16.9 ±0.11	13.8 ±0.10	11.5 ±0.17	14.9 ±0.05	17.1 ±0.10	15.7 ±0.06
Fatty acid composition (%) (v/w)	**1**	0.06 ±0.00^{a}	0.06 ±0.00^{a}	0.08 ±0.02^{a}	0.08 ±0.01^{a}	0.07 ±0.01^{a}	0.07 ±0.01^{a}	0.06 ±0.01^{a}	0.06 ±0.01^{a}
	2	7.45 ±0.00^{a}	6.87 ±0.04^{b}	6.92 ±0.03^{b}	7.67 ±0.08^{c}	8.18 ±0.09^{d}	7.39 ±0.01^{a}	7.48 ±0.03^{a}	7.33 ±0.02^{a}
	3	0.15 ±0.01^{ab}	0.19 ±0.00^{d}	0.17 ±0.01^{a}	0.15 ±0.01^{ab}	0.16 ±0.01^{a}	0.14 ±0.00^{b}	0.15 ±0.01^{ab}	0.12 ±0.00^{c}
	4	3.76 ±0.00^{b}	3.87 ±0.01^{a}	3.87 ±0.02^{a}	3.62 ±0.01^{e}	3.16 ±0.02^{d}	3.9 ±0.00^{c}	3.76 ±0.01^{b}	3.88 ±0.01^{a}
	5	15.33 ±0.01^{b}	17.14 ±0.02^{a}	17.14 ±0.01^{a}	16.17 ±0.02^{d}	15.61 ±0.03^{c}	16.24 ±0.02^{e}	16.44 ±0.03^{f}	16.84 ±0.04^{g}
	6	0.76 ±0.00^{bc}	0.82 ±0.00^{a}	0.83 ±0.01^{a}	0.82 ±0.01^{a}	0.79 ±0.01^{cd}	0.72 ±0.01^{e}	0.79 ±0.01^{d}	0.76 ±0.00^{b}
	7	71.55 ±0.01^{e}	70.15 ±0.01^{ab}	70.1 ±0.03^{a}	70.66 ±0.10^{c}	71.05 ±0.08^{d}	70.66 ±0.04^{c}	70.25 ±0.02^{b}	70.18 ±0.03^{ab}
	8	0.01 ±0.00^{a}	0.01 ±0.00^{a}	0.01 ±0.01^{a}	0.01 ±0.00^{a}	0.02 ±0.00^{b}	0.02 ±0.00^{b}	0.02 ±0.00^{b}	0.01 ±0.00^{a}
	9	0.42 ±0.00^{ab}	0.45 ±0.10^{b}	0.42 ±0.02^{a}	0.41 ±0.00^{a}	0.55 ±0.01^{e}	0.43 ±0.01^{ab}	0.52 ±0.01^{d}	0.38 ±0.00^{c}
	10	0.14 ±0.00^{a}	0.13 ±0.00^{a}	0.12 ±0.01^{a}	0.14 ±0.00^{a}	0.08 ±0.06^{b}	0.15 ±0.01^{a}	0.15 ±0.02^{a}	0.15 ±0.01^{a}
	11	0.21 ±0.01^{c}	0.18 ±0.01^{ad}	0.20 ±0.01^{abc}	0.17 ±0.01^{d}	0.20 ±0.01^{bc}	0.18 ±0.00^{abd}	0.20 ±0.01^{abc}	0.19 ±0.01^{abcd}
	12	0.01 ±0.00^{ab}	0.01 ±0.01^{ab}	0.02 ±0.00^{bc}	0.01 ±0.00^{ab}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.03 ±0.01^{c}	0.00 ±0.00^{a}
	13	0.03 ±0.00^{ab}	0.04 ±0.00^{a}	0.03 ±0.00^{ab}	0.02 ±0.00^{b}	0.04 ±0.00^{a}	0.03 ±0.00^{ab}	0.03 ±0.00^{ab}	0.03 ±0.00^{ab}
	14	0.01 ±0.01^{a}	0.00 ±0.00^{a}	0.01 ±0.01^{a}	0.00 ±0.01^{a}	0.01 ±0.01^{a}	0.00 ±0.00^{a}	0.01 ±0.01^{a}	0.00 ±0.00^{a}
	15	0.03 ±0.03^{a}	0.02 ±0.02^{a}	0.01 ±0.01^{a}	0.01 ±0.02^{a}	0.02 ±0.01^{a}	0.01 ±0.01^{a}	0.03 ±0.02^{a}	0.01 ±0.01^{a}
	16	0.03 ±0.01^{a}	0.03 ±0.01^{a}	0.03 ±0.01^{a}	0.02 ±0.00^{a}	0.03 ±0.02^{a}	0.02 ±0.01^{a}	0.03 ±0.01^{a}	0.02 ±0.00^{a}
	17	0.02 ±0.01^{a}	0.01 ±0.00^{a}	0.01 ±0.00^{a}	0.01 ±0.00^{a}	0.01 ±0.01^{a}	0.01 ±0.01^{a}	0.02 ±0.01^{a}	0.02 ±0.01^{a}
	18	0.03 ±0.00^{a}	0.03 ±0.02^{a}	0.03 ±0.01^{a}	0.03 ±0.01^{a}	0.03 ±0.01^{a}	0.03 ±0.00^{a}	0.03 ±0.01^{a}	0.02 ±0.01^{a}
Degree of unsaturation (%)		88.73 ±0.11	89.21 ±0.12	89.13 ±0.09	88.62 ±0.20	88.6 ±0.27	88.64 ±0.13	88.7 ±0.10	88.73 ±0.13

Note: 1 – myristic, 2 – palmitic, 3 – palmitoleic, 4 – stearic, 5 – oleic, 6 – vaccenic, 7 – linoleic, 8 – γ-linolenic, 9 – α-linolenic, 10 – octadecatetraenoic, 11 – cis-11-eicosenoic, 12 – arachidic, 13 – eicosatetraenoic, 14 – eicosapentaenoic, 15 – adrenic, 16 – docosapentaenoic n-6, 17 – docosapentaenoic n-3, 18 – docosahexaenoic, Mean ±SD of three determinations, ^{a} Means in a column, not followed by a common letter are significantly different according to Tukey's multiple range test (p <0.05).

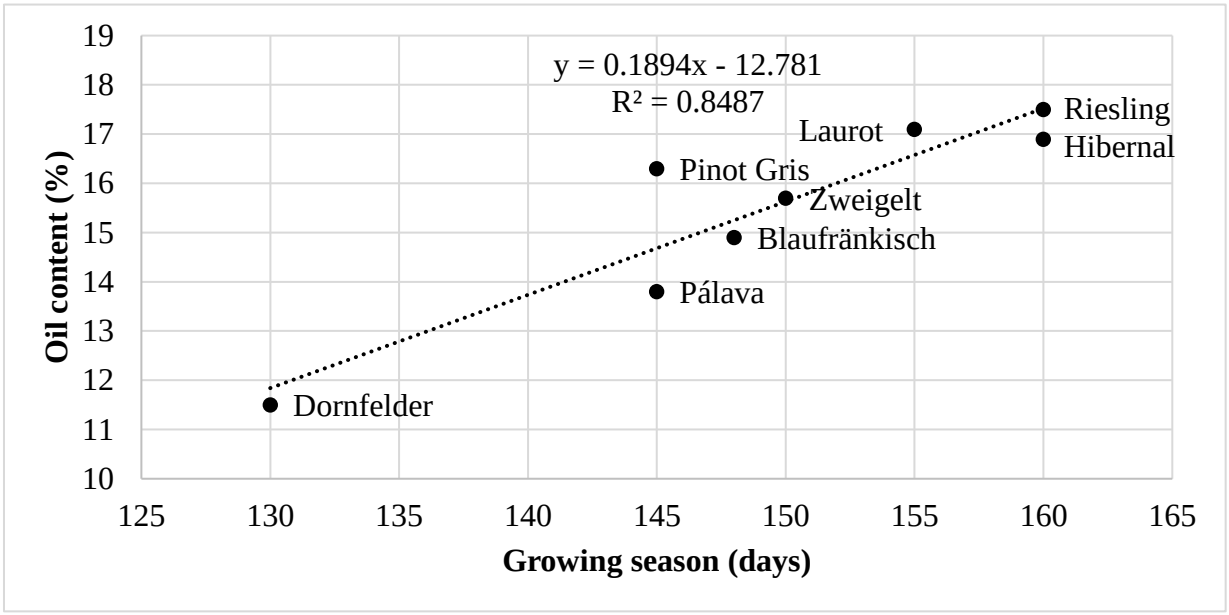

Figure 1 Influence of the growing season to the oil content in seeds.

Other author reached similar conclusions in his observations, and he states that oil content in different varieties is dependent on their maturity **(Ohnishi et al., 1990)**.

In Figure 1, there is stated a dependence between the length of growing season of individual varieties and the total oil content in seeds. Results clearly show lower oil content in seeds with a shorter growing season. There was found a very strong linear dependence in the samples, the coefficient of determination is $R^2 = 0.8487$.

Fatty Acid Composition

Fatty acid concentrations in evaluated oil samples ranged in different extent. The highest values were determined for linoleic acid 70.10 to 71.55%, oleic acid 15.61 to 17.14%, palmitic acid 6.87 to 8.18% and stearic acid 3.16 to 3.90%. Other fatty acids were represented in a relatively small amount in evaluated samples, e.g. myristic acid 0.06 – 0.08%, palmitoleic 0.12 – 0.19%, α-linoleic 0.38 – 0.55% etc. In oil samples from the varieties Blaufränkisch and Zweigelt, there was not determined arachidonic acid and eicosapentaenoic acid, in oil from the variety Riesling and Pálava, there was not determined eicosapentaenoic acid. The results of Tukey's honestly significant difference (HSD) showed differences between the evaluated oil samples, especially in the content of palmitic acid, stearic acid, oleic acid, 6-vaccenic acid and 7-linoleic acid, as stated in Table 1. The differences were statistically provable (p <0.05).

For example, **Barron et al. (1988)** and **Schuster (1992)** dealt with the evaluation of fatty acid content in grape seed oil. Their results imply, that in oil extracted from a mixed sample of seeds, there are mostly represented palmitic, stearic, oleic, and linoleic acids. **Ohnishi et al. (1990)** dealt with the issue of representation of fatty acids in varietal oils. In analyses of oils from five grape cultivars, he determined that the representation of palmitic acid is at the level 6.7 to 8.9%, stearic 1.1 to 5.3%, oleic 9.7 to 17.5% and linoleic 69.2 to 80.5%. He determined low values under the level of 0.1% for palmitoleic and linolenic acid. **Crews et al. (2006)** state that in grape seed oil, there is mostly represented linoleic acid (58 – 78%) and oleic acid (10 – 20%). **Morin (1996)** and **Firestone (1999)** also state that grape seed oil is on average composed of 90% poly- and monounsaturated fatty acids, which are responsible for its value as nutritive edible oil, particularly of linoleic acid (58 – 78%) followed by oleic acid (3 – 15%) and minor amounts of saturated fatty acids (10%). Similar results are shown also by the evaluated samples, as stated in Table 2.

Ohnishi et al. (1990) state that the fatty acid composition of grape seed oil is similar to that of sunflower, or safflower oil. Sunflower oil generally comprises of 44 – 75% linoleic, 14 – 35% oleic, 3 – 6% palmitic and 1 – 3% stearic acid and safflower oil generally comprises of 73 – 79% linoleic, 13 – 21% oleic, 3 – 6% palmitic and 1 – 4% stearic acid **(Crews et al., 2006)**.

The degree of unsaturation in the grape seed oil (Table 1) was over 88%, coming from unsaturated fatty acids. **Baydar et al. (2007)** state that grape seed oils are rich in oleic and linoleic acids and the degree of unsaturation in the oils is over 85%. High levels of unsaturation play an important role in lowering of high blood cholesterol and also in the treatment of atherosclerosis **(Gey 1993)**. **Kinsella et al. (1993)** also states that fatty acids such as omega-3 and omega-6 significantly decrease the concentration of LDL cholesterol and their higher consumption significantly decreases the risk of myocardial hearth attack and sudden death. **Bagchi et al. (2003)** state that this positive influence is probably caused not only by the decrease of cholesterol in blood, but also by lowering of blood coagulation.

Poly-unsaturated fatty acids such as linoleic and linolenic are essential for a human body and grape seed oil may be their significant source. Grape seed oil was rather poor in linolenic acid.

To reduce smell and undesirable taste of edible oils, there is beneficial low representation of linoleic acid. **Baydar and Akkrut (2001)** state that lower content of linoleic acid positively influences shelf-life of oil. The reason is the fact that linolenic acid is simply oxidized due to having three double bonds on its hydrocarbon chain, the stability or shelf-life of an oil rich in linolenic acid would be too short.

Table 2 Fatty acid composition (%) (v/w) according to grape variety.

Cultivar	Saturated	Unsaturated	Poly-unsaturated	n-6	n-3	n-3/n-6
Pinot Gris	11.273	16.454	72.274	71.629	0.645	0.009
Riesling	10.793	18.326	70.882	70.212	0.670	0.010
Hibernal	10.877	18.345	70.793	70.174	0.622	0.013
Pálava	11.373	17.304	71.319	70.711	0.608	0.009
Dornfelder	11.398	16.767	71.836	71.122	0.714	0.010
Blaufränkisch	11.360	17.281	71.360	70.706	0.655	0.009
Laurot	11.304	17.582	71.123	70.368	0.766	0.017
Zweigelt	11.270	17.915	70.815	70.219	0.596	0.008

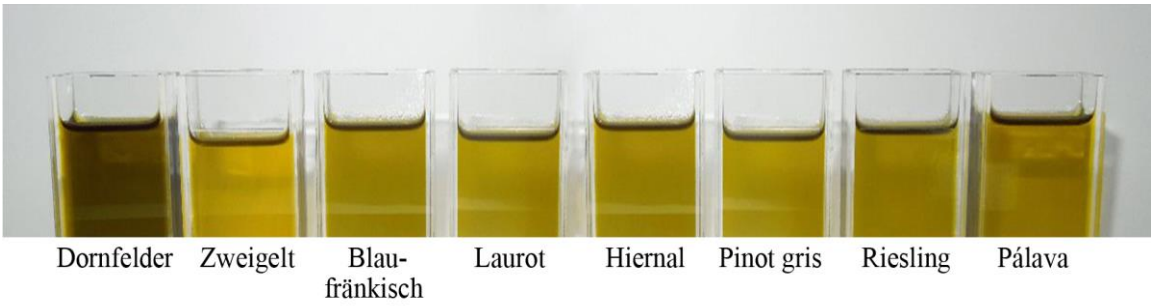

Figure 2 Colours of tested grape seed oils.

CONCLUSION

The grape seeds of eight grape cultivars (Riesling, Pinot Gris, Pálava, Hibernal, Dornfelder, Blaufränkisch, Zweigelt and Laurot) were evaluated in terms of oil content and oil quality properties including fatty acid composition. The oil content was found to be different for each cultivar, which ranged from 11.5% (Dornfelder) to 17.5% (Riesling). In the analysed samples, there were proved statistically significant differences in fatty acid composition. Saturated fatty acid values were less (10.79 – 11.40) than the values of monounsaturated fatty acids and polyunsaturated fatty acids in all evaluated oil samples. Among the identified fatty acids, linoleic acid was the predominant fatty acid and followed by oleic acid, palmitic acid and stearic acid in all varieties. Following the conducted analyses, there can be seen that the quality properties of fatty acids are markedly dependent on their degree of saturation. Unsaturated fatty acids have a lower melting point than saturated fatty acids. Grape seeds as a large by-product from wine production may be evaluated as a source of high-quality vegetable oil, which can be used as a food supplement to improve the nutritional value of the human diet.

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