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Fatty Acid Composition of Grape Seed Oil as Affected by Grape Variety and Extraction Solvent

Jianmei Yu[1]*, Ivy Smith[1], John Carver[2] and Bryce Holmes[2]

[1]*Department of Family and Consumer Sciences, North Carolina A&T State University, Greensboro, NC, USA*

[2]*Department of Natural Resource and Environmental Design, North Carolina A&T State University, Greensboro, NC, USA*

***Corresponding Author:** Jianmei Yu, Department of Family and Consumer Sciences, North Carolina A&T State University, Greensboro, NC, USA.

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Abstract

Grape seeds are valuable by-product of grapes from wine industry. In addition to health promoting polyphenols, grape seeds contain significant amount of oil. In this study, grape seed oils (GSOs) were extracted from the seeds of two Muscadine cultivars and two Cabernet cultivars using hexane and chloroform-methanol. The oil yields and total polyphenol contents of the crude oil were quantified. The fatty acid composition of each GSO sample was determined by GC. Results show that Muscadine Nobel seeds had lowest total oil yield regardless the extraction solvents. Major fatty acids in GSOs were linoleic (C18:2), oleic (C18:1), palmitic (C16:0) and stearic acids (C18:0). Hexane extracted oils showed higher C16:0, C18:0, C18:2, but lower C18:1 contents than chloroform-methanol extracted oils. Total unsaturated fatty acid contents were 83.34 - 85.93 for hexane extracted oil 85.71 - 88.84% for chloroform-methanol extracted oils, respectively, depending on the grape cultivars. Linoleic acid dominated the unsaturated fatty acids in all samples. The high unsaturated fatty acid content makes GSO a good choice for food products that need to be in the liquid form at temperature. Total polyphenol contents of GSO also varied with extraction solvents and is cultivar dependent. The results indicate that fatty composition of different grape seed varies only slightly, but is influenced significantly by extraction solvent due to the polarity difference of different fatty acid. Therefore, when comparing fatty acid composition and antioxidant content of oils from different materials, it is important to make sure that all oil samples are extracted by same solvent and under same extraction condition.

Keywords: *Grape Seed Oil; Extraction Solvent; Oil Yield; Fatty Acid Composition; Polyphenol Contents*

Abbreviations

GSO: Grape Seed Oil; Mus: Muscadine; Cab: Cabernet; FFA: Free Fatty Acid; FAMEs: Fatty Acid Methyl Esters; GAE: Gallic Acid Equivalent; SFA: Saturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; PUFA: Polyunsaturated Fatty Acid

Introduction

Wine grapes contain much more seeds than table grapes. Wine grape seeds are end up in the grape pomace which counts for 20 - 25% of grapes crushed for wine making and is typically composted or disposed. The grape seed content in dry grape pomace is in the range of 36 - 82% depending on the variety of grape [1]. In addition to health promoting polyphenols, grape seeds contain significant amount of oil. Grape seed oil (GSO) has become popular in recent years and have been used in cosmetic products and dietary supplement.

The nutritional quality and the physical/chemical properties of a food oil is determined by its fatty acid composition and the presence of antioxidants. Many studies found that the most abundant oil of grape seed oil was linoleic acid (C18:2) followed by oleic acid (C18:1), but the percentage of linoleic acid and oleic acid varied from 66% (Ruby grape seed oil) to 75% (Concord grape seed oil) depending on grape variety [2,3]. Oil rich in polyunsaturated fatty acids such fish oil and flax seed oil has been reported to have many health benefits [4], but are more susceptible to oxidation [5]. The fatty acid compositions could be a predictor for the oxidation stability of the vegetable oils at the early stage of oil oxidation, but not for those at a later stage of oxidation [5].

In addition to the esterified fatty acids, free fatty acids (FFA) are naturally present in low amounts in vegetable oils as a product of hydrolytic degradation of triglycerides. They are more susceptible to autoxidation than esterified fatty acids in bulk oils. The presence of FFAs in the oil will adversely affect the flavor and stability of the oil. FFAs were considered as strong prooxidants in both bulk and emulsified oils, and the prooxidant effect of FFAs was dependent on fatty acid type with lipid oxidation rates being in the order of linolenic < linoleic < oleic [6,7].

The antioxidant and fatty acid compositions of grape seed oil, thus its nutritional and cosmetic properties may be significantly affected by the grape variety and maturity [8-10], oil extraction methods and degree of refining [11,12]. Grape seeds also contain non-phenolic antioxidants such as tocopherols, carotenoids and phytosterols [2,9,13-15]. The quality and stability of food oils are influenced by the presence of some minor components, such as free fatty acids, tocopherols, phospholipids, trace metals and waxes which have pro- or antioxidant properties. Most of these minor components are removed during oil refining process to improve the stability but small quantity remains in the oil [16,17]. Grape seed oil may also contain significant amount of hydrophobic polyphenols which could contribute to the stability of the oil. Therefore, it is important to assess the fatty acid composition, FFAs and antioxidant contents of grape seed oil from different varieties of grapes.

The most proper solvent for grape seed oil extraction was reported to be propane because oil samples extracted with propane present a smaller amount of free fatty acids [11], but propane cannot extract polar compounds such as phytosterols. Although chloroform-methanol can extract both non-polar and polar fatty acid, the use of chloroform may result in health risk because chloroform is carcinogenic. In addition to solvent extraction, cold expeller press is another common oil extraction method which is a better method to produce food grade GSO because organic solvent is not used.

In this study, the effects of extraction solvents on oil yield, free fatty acid content, fatty acid composition, and total polyphenol content of GSO from the seeds of four grape cultivars, namely, Muscadine Nobel, Muscadine Scuppernong, Cabernet Franc and Cabernet Sauvignons were evaluated. The reason of selecting these four cultivars is that Muscadine Noble and Carlos are the two most popular native wine grapes in the United States, while Cabernet Sauvignon and Franc are two of the popular *Vitis Vinifera* wine grapes worldwide. The results provided useful information for further study of GSO and provided insights for the application of GSO.

Materials and Methods

Preparation of grape seeds for oil extraction

Freshly pressed grape pomaces from four grape cultivars, Muscadine Nobel (Mus-Noble), Muscadine Scuppernong (Mus-Scup), Cabernet Franc (Cab-Franc) and Cabernet Sauvignons (Cab-Sauv), were obtained from two North Carolina wineries. The pomaces were spread as thin layer in trays and dried at room temperature (23°C) in a fume hood to final moisture content about 5%. Grape seeds were manually separated from dry grape pomace and ground into powder using a high speed blender (Fisher Scientific, Atlanta, GA) and the portion passed 40 mesh standard sieve was stored at -20°C and used for oil extraction.

Extraction of grape seed oil (GSO)

Extraction GSO using chloroform-methanol: This solvent system allows for extraction of both polar lipids (such as phospholipid, cholesterol) and non-polar lipids (mainly triglycerides and free fatty acids) [18]. Briefly, 30 ml of water was mixed with 10.00g of dry ground grape seeds (40 - 100 mesh) in a 250 ml flask, then 100 ml of 1:1 mixture of chloroform and methanol was added and stirred at room temperature for 10 minutes. The grape seed residual was removed by filtration through Whatman No. 2 filter paper in a Buchner funnel with slight suction (low vacuum) to ensure maximum solvent recovery. The residual was washed with 20 ml 1:1 (v/v) chloroform-methanol. Combined filtrate was transferred into a separation funnel. The lipid in the chloroform layer was collected and then passed through a 2.5-cm thick layer of anhydrous sodium sulfate and collected in a flat bottom evaporation flask with known weight. The solvent was removed using an R-200 rotary evaporator (Büchi Corporation, Switzerland) under vacuum, and the lipid yield was recorded. The lipid was transferred into an amber glass vial and stored at 4°C for analysis. The oil extraction was conducted in triplicate.

Extraction of GSO by hexane: The ground grape seed flours were mixed with hexane at the ratio of 1:10 (v/w) and stirred on a magnetic multiple stir plate for one hour at room temperature. The mixture was then filtered through Whatman No. 2 filter paper and the oil residue was extracted one more time under same condition. The combined extracts were transferred to an evaporation flask with known weight and concentrated using an R-200 rotary evaporator at 40°C to remove hexane which was recovered and used for next extraction. The oil yield was recorded and the oil was transferred into a glass vial and stored at 4°C for analysis. The oil extraction was conducted in triplicate.

Polyphenol extraction and analysis

Polyphenols in the GSO were extracted using methanol. Briefly, 1 ml of methanol was added in 0.5 ml of GSO in a glass vial. After vortex, the mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant (upper layer) was collected using glass transfer pipette and analyzed for total polyphenol by Folin-Ciocalteu method [19] and expressed as gallic acid equivalent (µg GAE) per gram crude oil. The polyphenol extraction and analysis was conducted in triplicate.

GSO purification

An amount of 0.25g of GSO extract was weighed into a vial, and 10.0 ml of 2:1 Chloroform- Methanol was added. Sample was vortexed for 30 seconds, allowed to settle for 5 minutes, then centrifuged for 10 minutes at 3000 rpm. The top layer was drawn off and 3.0 ml of the chloroform/methanol layer was pasted through a supelclean LC-NH2 SPE cartridge (Supelco, Bellefonte, PA, USA). The fatty acids were eluted off the LC-NH_2 column using 2% acetic acid in diethyl ether and collected in a sample vial for analysis on GC.

Analysis of free fatty acids (FFA) in GSO by gas chromatography (GC)

The FFA composition of purified fractions was determined by a Thermo-Fisher Trace Ultra GC with a TriPlus Auto sampler (Ashville, NC, USA) and a Flame Ionization Detector (FID). Column for FFA separation was Nukol (15m x 0.53 mm ID x 0.50 µm film thickness). The sample injection volume was 1 µl. Helium was used as carrier Gas (7 ml/min, constant flow). Both injector and detector temperatures were 220°C. Oven Temp was started at 100°C, ramp 8°C/min to 220°C and held at 220°C for 25 minutes. The individual fatty acid concentration was calculated according to the standard curves developed using GC-463 standard mix (Nu Check, Elysian, MN, USA) under the same GC conditions.

Analysis of fatty acids of triglycerides in GSO by GC

Fatty acids of triglycerides in GSO were converted into fatty acid methyl esters (FAMEs) by methylation before GC analysis. The purpose of methylation was to convert the non-volatile triglycerides into volatile FAMEs. Briefly, 0.25g of purified grape seed oil extract was

weighed into a vial and 1.0 ml of tetrahydrofuran was added at room temperature with 1.0 ml of methanolic 1M KOH then vortexed for 30 seconds. Samples were allowed to settle for 1 minute and then 1.0 ml of 14.0% boron trifluoride was added and vortexed for 1 minute. The solution was then heated in a 100°C water bath for 15 minutes. After cooled to room temperature, 0.5 ml of saturated NaCl, 1.0 ml of heptadecanoic acid (1 mg/ml) and 1.0 ml of isooctane were added. The mixture was vortexed for 30 seconds, allowed to settle and the top layer containing FAMEs was removed for GC analysis.

The FAMEs were determined by Thermo-Fisher Trace Ultra GC@ with a TriPlus Auto Sampler and an FID. DB-23 column (60m, 0.18 mm ID, 0.14 μm film thickness, Nucheck,) was used for fatty acid separation. Carrier Gas was helium at 400 psi constant pressure. The injector temperature was 220°C and detector temperature was 230°C. The oven temperature was programmed as following: starting 45°C and holding for 2 minutes, ramp 10°C/min to 80°C and holding for 2 minutes, ramp 8°C/min to 180°C and holding for 2 minutes and then ramp 2°C/min to 240°C and holding for 5 minutes. The individual fatty acid concentration was calculated according to the standard curves developed using GC-463 standard mix under the same GC conditions.

Data analysis

The oil yield and TPC data were expressed as mean ± standard deviation. The comparison of oil yield and TPC of GSO from different grape seeds and by different extraction solvents were conducted by post-ANOVA multiple range comparison using SAS 9.4. (SAS Institute, Cary, NC USA).

Results and Discussions

Effects of solvent and grape cultivar on GSO yield

The oil yield of grape seeds was significantly affected by extraction solvent ($P < 0.01$) and grape cultivar ($P < 0.001$) (Figure 1). The lowest oil yield (7.4%) was obtained from the seeds of Muscadine Nobel grapes, while the highest oil yield (12.8%) was obtained from the seeds of Cabernet Franc grapes regardless of extraction solvent. The hexane extraction resulted in slightly higher oil yield than chloroform-methanol extraction for same type of grape seeds except Cabernet Franc seeds. This might be caused by the lower hydrophobicity of chloroform-methanol. The variation of oil yield with extraction solvents has been reported by many researchers who found that heptane and hexane extraction resulted in higher oil yield than acetone and ethanol [9,20,21]. However, for the same solvent, the GSO yield depends on grape variety which determines the oil content of the seeds. Other extraction parameters such as particle size, temperature and extraction time may also affect oil yield.

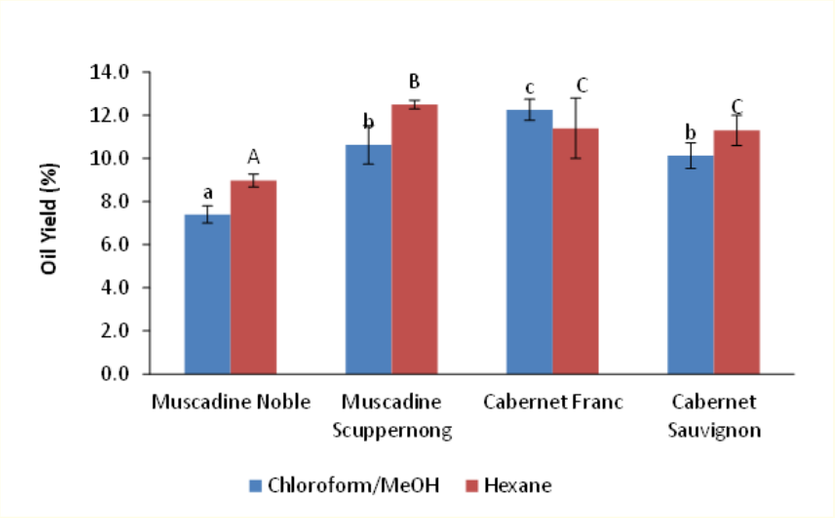

Figure 1: *Oil yields of grape seeds from different grape varieties as affected by extraction solvents (for same cultivar, data with different labels are significantly different at $P < 0.01$).*

Total polyphenol contents (TPCs) of grape seed oils

Figure 2 shows that the TPCs of GSOs varied with grape cultivars greatly and was significantly affected by extraction solvents. No matter what solvent was used, The TPCs of GSOs from Muscadine Noble seed and Cabernet Sauvignon seeds were higher than those of Muscadine Carlos and Cabernet Franc seed oils. For same grape seeds, hexane extracted oil had significantly higher TPC content than chloroform-methanol extracted oils ($P < 0.05$). The TPC of Muscadine GSO obtained in this study (401 - 590 µg GAE/g oil) is comparable to that of GSO obtained by cold pressed [2] and higher than that of extra virgin olive oil which was reported to contain 100 - 300 mg/kg [22]. The main polyphenols identified in GSO were reported to be catechins, epicatechins, trans-resveratrol, and procyanidin B1 [23]. Although considered hydrophilic, the solubility of these phenolic compounds in water is very low. Higher TPC in hexane extracted GSO indicates that the hydrophobicity of phenolic compounds in GSO is higher than their hydrophilicity. They may serve as antioxidants to slow down the oxidation of the GSO if they are not removed during oil refining.

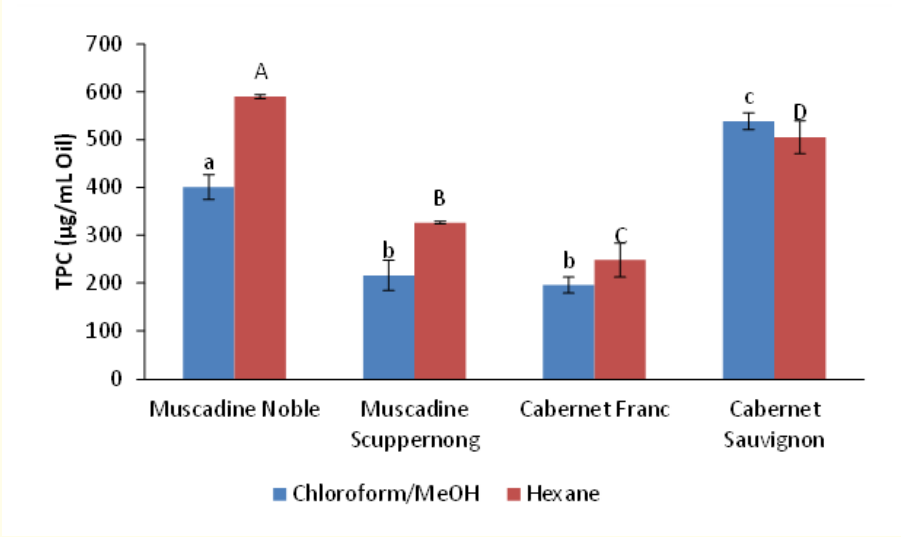

Figure 2: *Polyphenol contents of oils extracted from seeds of different grape cultivars as affected by extraction solvents (for same cultivar, data with different labels are significantly different at $P < 0.05$).*

Free fatty acid (FFA) composition and quantity of GSO

Table 1 show that the major FFAs in the chloroform-methanol extracted GSO were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and C20:0 regardless of grape variety and extraction solvent. Among four types of grape seeds, Muscadine Noble and Scuppernong seeds had lower FFA content than Cabernet Franc and Sauvignon seeds. For the seeds of same grape variety, chloroform-methanol extraction resulted in much higher amount of FFAs than hexane extraction. The highest FFAs was found in the oil of Cabernet Sauvignon seeds (3,622 mg/kg oil or 0.36%). The presence of unsaturated FFAs, particularly, C18:2 and C18:3, in the oil will result in significantly reduced oxidative stability of the oil [7,24,25]. Although oil refining process will remove most of the FFAs, it will also remove other important components such as, vitamin E, phenolic compounds and phytosterols [16,22,26].

Fatty acid (FA) composition of triglycerides of GSO

Short-chain and medium-chain FAs were not detected in the triglycerides of GSOs extracted in this study although trace amount of medium-chain FAs (0.01 - 0.06%) such as C8:0, C12:0 and C14:0 were reported [22]. The major FAs of GSO were long chain FAs and the

most abundant FA was C18:2 followed by C18:1, C16:0 and C18:0 regardless grape cultivar and extraction solvent (Table 2). The C18:2 accounted for 70.90 - 77.38% of total fatty acid depending on the variety of grape and extraction solvent with higher C18:2 and total unsaturated fatty acids in Muscadine GSO than in Cabernet GSO. Muscadine GSOs also had higher C18:1 than Cabernet GSOs no matter what solvent was used. The crude GSOs extracted in this study has lower total monounsaturated fatty acid (MUFA) but higher total polyunsaturated fatty acid (PUFA) than all other reported edible plant oils except safflower seed oil and walnut oil [27,28]. The total unsaturated FA content of Muscadine and Cabernet GSOs were 85.1 - 88.8% and 83.34 - 86.3%, respectively. Major saturated FA of GSOs were palmitic acid (C16:0) and stearic acid (C18:0). Total saturated FA varied with grape cultivars and extraction solvents. Among all grape seeds analyzed, Muscadine Nobel and Cabernet Sauvignon seeds showed lowest and highest saturated fatty acid, respectively. The saturated FA contents of oils extracted using chloroform-methanol and hexane were in the range of 11.16 - 14.61% and 14.85 - 16.66, respectively. Overall, the GSOs extracted by chloroform-methanol had higher unsaturated FAs while GSOs extracted by hexane showed higher saturated FAs for all grape varieties.

	GSO extracted by chloroform-methanol				GSO Extracted by Hexane			
Fatty acid	**Mus* Nobel**	**Mus Scuppernong**	**Cab† Franc**	**Cab Sauvignon**	**Mus Nobel**	**Mus Scuppernong**	**Cab Franc**	**Cab Sauvignon**
C14:0	5.00	7.80	9.00	8.00	5.08	2.78	4.25	3.32
C16:0	346.00	384.00	400.00	712.00	32.66	13.91	30.83	20.45
C16:1	-	-	-	-	20.84	0.79	0.00	0.81
C18:0	330	430	380	659	17.45	8.25	13.77	10.4
C18:1	211.00	204.00	190.00	343.00	34.65	13.28	28.07	17.35
C18:2	493.00	371.00	520.00	1,490.00	150.51	42.35	84.3	62.78
C18:3	285.00	270.00	190.00	359.00	2.84	0.95	1.56	0.79
C20:0	49.00	79.00	430.00	51.60	14.06	10.87	10.75	10.19
Total	1719.00	1,745.80	2119.00	3,622.60	278.09	93.18	173.53	126.09

Table 1: *Free fatty acid compositions of grape seed oils extracted by chloroform- methanol and hexane.*
**Mus-Muscadine, †Cab-Cabernet.*

	Chloroform-Methanol Extracted Oils				Hexane Extracted Oils			
Fatty acid (%)	**Mus* Nobel**	**Mus Scuppernong**	**Cab† Franc**	**Cab Sauvignon**	**Mus Nobel**	**Mus Scuppernong**	**Cab Franc**	**Cab Sauvignon**
C14:0	<0.01	0.02	0.06	0.05	0.04	0.03	0.08	0.07
C16:0	6.34	6.88	8.80	8.51	9.39	9.39	10.00	10.38
C16:1	0.12	0.06	0.09	0.07	0.07	0.04	0.11	0.09
C17:0	0.11	0.05	0.05	0.04	.	.	.	.
C18:0	4.50	6.18	5.18	4.89	5.30	5.30	5.85	6.04
C18:1	14.20	11.91	14.51	13.97	7.50	7.50	6.52	7.09
C18:2	74.21	74.53	70.90	72.07	77.38	77.37	77.08	75.90
C18:3	0.31	0.15	0.20	0.21	0.20	0.17	0.22	0.26
C20:0	0.21	0.19	0.17	0.16	0.13	0.20	0.18	0.17
Total SFA	11.16	13.30	14.21	13.59	14.85	14.92	16.10	16.66
MUFA	14.32	11.98	14.61	14.04	7.57	7.54	6.63	7.18
PUFA	74.52	74.69	71.10	72.28	77.58	77.54	77.30	76.16
Total Unsaturated	88.84	86.66	85.71	86.32	85.15	85.08	83.93	83.34
Total	100.00	99.98	99.98	99.97	100.00	100.00	100.00	100.00

Table 2: *Fatty acid composition of triglyceride GSO from different varieties of grapes and the effects of extraction solvents.*
Mus-Muscadine, †Cab-Cabernet.

Conclusion

This study indicates that fatty acid composition of GSO is cultivar dependent and varies with the extraction solvent. Regardless of cultivars, major fatty acids in GSOs were linoleic (C18:2), oleic (C18:1), palmitic (C16:0) and stearic acids (C18:0). Hexane extracted oils showed higher C16:0, C18:0, C18:2, but lower C18:1 contents than chloroform-methanol extracted oils. Hexane extracted GSO also had higher total unsaturated fat. The results indicated that fatty composition of different grape seed varies only slightly, but is influenced significantly by extraction solvent due to the polarity difference of different fatty acid. Therefore, when comparing fatty acid composition of oils from different materials, it is important to make sure that all oil samples are extracted by same method and under same extraction condition. The high PUFA content makes GSO a good choice for salad dressing because it will not solidify even at refrigeration temperature. GSO is also rich in polyphenols that will serve as natural antioxidants in oil. However, the high PUFA and free FFA contents also make GSO's stability a problem because the oil rich in PUFA is more prone to oxidation. In addition, although unsaturated fat has been reported to be healthier than saturated fat, particularly to the cardiovascular disease (CVD), the benefits of replacing SFAs with PUFAs are questionable because there is no evidence that a lower intake of SFA can prevent CVD and a high intake of PUFAs without specification may result in a high intake of omega-6, which is associated with many adverse health effects (Ravnskov., *et al*. 2014). Therefore, further studies on the oxidative stability and health benefit of GSOs are necessary.

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Bibliography

1. Yu J. "Thermal stability of major classes of polyphenols in skins, seeds and stems of grape pomace". In "Grapes: Production, Phenolic Composition and Potential Biomedical Effects" José S. Câmara (Ed). Nova Science Publishers Inc. Hauppauge, NY (2014).

2. Lutterodt L., *et al*. "Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours". *Food Chemistry* 128 (2011): 391-399.

3. Orsavova J., *et al*. "Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids". *International Journal of Molecular Science* 16 (2015): 12871-12890.

4. Briggs MA., *et al*. "Omega-3 polyunsaturated fatty acids and health". In: Food Lipids-Chemistry, Nutrition and Biotechnology (Casimir C. Akoh, Editor): 603-626.

5. Yun JM and Surh J. "Fatty acid composition as a predictor for the oxidation stability of Korean vegetable oils with or without induced oxidative stress". *Preventive Nutrition and Food Science* 17 (2012): 158 -165.

6. Paradiso VM., *et al*. "Effects of free fatty acids on the oxidative processes in purified olive oil". *Food Research International* 43 (2010): 1389-1394.

7. Waraho T., *et al*. "Impact of free fatty acid concentration and structure on lipid oxidation in oil-in-water emulsions". *Food Chemistry* 129 (2011): 854-859.

8. Rao PU. "Nutrient composition of some less-familiar oil seeds". *Food Chemistry* 50 (1994): 378-382.

9. Beveridge THJ., *et al*. "Yield and composition of grape seed oils extracted by supercritical carbon dioxide and petroleum ether: varietal effects". *Journal of Agricultural and Food Chemistry* 53.5 (2005): 1799-1804.

10. Llobera A and Canellas J. "Dietary fiber content and antioxidant activity of Manto Negro red grape (Vitisvinifera): pomace and stem". *Food Chemistry* 101 (2007): 659-666.

11. Passos CP., *et al*. "Enhancement of the supercritical fluid extraction of grape seed oil by using enzymatically pre-treated seed". *The Journal of Supercritical Fluids* 48(2009): 225-229.

12. Dos Santos Freitas L., *et al*. "Extraction of grape seed oil using compressed carbon dioxide and propane: extraction yields and characterization of free glycerol compounds". *Journal Agricultural and Food Chemistry* 58 (2008): 2558-2564.

13. Baydar NG and Akkurt M. "Oil content and oil quality properties of some grape seeds". *Turkish Journal of Agriculture and Forestry* 25 (2001): 163-168.

14. Bravi., *et al*. "Improving the extraction of α-tocopherol-enriched oil from grape seeds by supercritical CO2. Optimization of the extraction conditions". *Journal of Food Engineering* 78 (2007): 488-493.

15. Ren Y. "Oxidative stability of omega-3 polyunsaturated fatty acids enriched eggs". *Journal of Agricultural and Food Chemistry* 61 (2013): 11595-11602.

16. Pal US., *et al*. "Effect of refining on quality and composition of sunflower oil". *Journal of Food Science and Technology* 52 (2015): 4613-4618.

17. Lucci P., *et al*. "Effect of the refining process on total hydroxytyrosol, tyrosol, and tocopherol contents of olive oil". *Foods* 9.3 (2020): 292.

18. Bligh EG and Dyer WJ. "A rapid method of total lipid extraction and purification". *Canadian Journal of Biochemistry and Physiology* 37(1959): 911-917.

19. Singleton VL., *et al*. "[14]Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent". *Methods in Enzymology* 299 (1999): 152-178.

20. Ghazali Q and Yasin NHM. "The effect of organic solvent, temperature and mixing time on the production of oil from Moringa oleifera seeds". *IOP Conf. Series: Earth and Environmental Science* 36 (2016): 012053.

21. Efthymiopoulosa I., *et al*. "Influence of solvent selection and extraction temperature on yield and composition of lipids extracted from spent coffee grounds". *Industrial Crops and Products* 119 (2018): 49-56.

22. Van Hoed V. "Phenolic compounds in seed oils". *Lipid Technology* 22 (2010): 247-249.

23. Garavaglia *et al*. "Grape seed oil compounds: biological and chemical actions for health". *Nutrition and Metabolic Insights* 9 (2016): 59-64.

24. Frega N., *et al*. "Effects of free fatty acids on oxidative stability of vegetable oil". *Journal of American Oil Chemists Society* 76 (1999): 325-329.

25. De Leonardis A., *et al*. "Influence of free fatty acid content on the oxidative stability of red palm oil". *RSC Advances* 6 (2016): 101098-101104.

26. García A., *et al*. "Effect of refining on the phenolic composition of crude olive oils". *Journal of American Oil Chemists Society* 83(2006): 159-164.

27. Dubois V., *et al*. "Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential". *European Journal of Lipid Science and Technology* 109 (2007): 710-732.

28. Kostik V., *et al*. "Fatty acid composition of edible oils and fats". *Journal of Hygienic Engineering and Design* 4 (2013): 112-116.

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