

Utilization of grape seed oil and grape seed flour in food industry

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ABSTRACT.

Grape production is considered to be one of the most important agro economic activities in the world, with more than 67 million tons of grapes (*Vitis vinifera*) produced globally in 2012, about 22 million tons of them produced in the European Union [16]. However, a large volume of winery wastes remains unexploited every year on an international level.

This study aims at exploring various ways of winery waste (seeds, stems and peels) usage; specifically its goal is to provide alternative solutions on the use of grape seeds. The objective is to encourage wineries to apply value adding technologies in order to reduce their waste generation and disposal. Moreover, this research provides alternative solutions to diminish wineries' environmental impact and introduces to them additional sources of income through the use of grape seed related products in food industry, cosmetology, as well as in medicine.

Grape seeds from Greek grape varieties that were used in this study were naturally dried in the sun and cleaned. Grape seed oil and grape seed flour were produced from mechanical extraction that preserves the natural structure of oil.

Findings about the grape seed's oil (acidity, fat, peroxide value, absorption spectroscopy) and grape seed flour's profile (humidity, ash, total crude fiber, fat) were gathered in order to obtain more solid facts. Our results showed, among others, that grape seed oil is a good source of fatty acid. Grape seed flour demonstrated to be a good source of fiber (46%-48%) and could be an ingredient to be used in various foods.

The study proved that grape seeds have the ability to be reused; their oil and flour can be a main ingredient in other food products with positive effects on human health.

Introduction

During the red and the white vinification, a large number of by products is produced, some of them are further used and other not. In Greece, it is estimated that the annual production is about 525,000 tons of grapes which consequently leads to 142,000 tones of winery waste [16]. It is estimated that 100 kg fresh marc are constituted from 30 kg of fresh pulp, 25 kg of fresh seeds and 20 kg stalks [27].

According to Torres et al. [47], 13% of the weight of the grapes processed in winemaking is used for by products. The peel and the grapes seeds are the main by-products that can be used either to produce another product (distillate oil) or to be used as compost for the production of alcohol or create a new type of human food.

Pulp can be composted after mixing with other minerals and used as fertilizer a process already taking place in France [37]. After the extraction of grape seed oil, grape seed powder is usually used as animal food. The extracts of grape marc can be fermented with special crops and produce high added value and purity substances such as polysaccharide Pullulan or produce alcohol [25].

Recent studies have shown that winery by-products may negatively affect the environment by presenting toxicity to crops [32, 33] and wetlands [4]. Grismer et al. [22] focused on wastewater wineries which were considered responsible for the contamination of groundwater resources.

Using the marcs with the composting process, a substrate for plants [20, 31, 35, and 36], and a substrate for the cultivation of mushrooms [37] and bleaching substances [35] have been produced.

Through the process of fermentation and by using different microorganisms with different treatment (hydrolysis or not), lactate [40, 41], bioactive emulsifiers [42], hydrolytic enzymes [13] bio-ethanol [43], while the extraction process tannins [26], polyphenols [10, 18, 39, 48] are produced.

Furthermore, grape seed powder has been used in food as a basic element in fettuccini pasta [44] and in frankfurters [34]. According to the already mentioned studies, grape seed powder can improve the nutritional profile (increased the protein and dietary fibre content and oxidation was minimized) and improve the sensorial acceptability of the products. In Greece, the majority of by products is used for the production of spirits, pure alcohol, for compost production with the aerobic decomposition (biodegradation) or discarded as useless.

After the appropriate treatment, by products and residues of wine are able to form a new economic resource while meeting the environmental protection standards. Moreover, their use could reduce the production cost of the winery, the reduction of existing environmental footprint, but also to create new jobs.

2. Materials and methods

2.1 Grape seed samples

Five greek grape varieties were selected, one red, Mavro Arahovis and four white, Savvatiano, Asyrtiko, Malagousia and Roditis from three different wineries in Greece. After harvest, the grapes were dried for seven days in open air, they were weighted and the seeds and skins were selected and separated by hand. The seeds were stored in glass containers.

2.2 Grape seed oil extraction and grape seed powder

The production of grape seed oil was done mechanically, which is considered to be the safer way as the physical structure of the components is maintained. The grape seed powder results from the extraction of the grape seed oil.

The oil was extracted with an Italian electric hydraulic press OMCN, model 204 / RE with capacity 150TN. This force was achieved at a pressure of 400 bar hydraulic piston. The amount of seeds for every batch was compressed 0,8lt. The oil flow started at approximately 100 bar pressure hydraulic press.

The oil was decanted after seven days and stored for further analysis.

Grape Variety	Net weight of grape seeds (g)	Net weight of grape seed oil (g) Yield (%)	Net weight of grape seed powder (g) Yield (%)
Mavro Arahovis	1.518	71 4,6	1.242 81
Savvatiano	800	40 5	582 73
Asyrtiko	1.570	126 8	1.163 74
Malagousia	2.770	160 5,8	1.556 56
Roditis	1.300	100 7,7	810 62

Table 1. The above table summarizes the quantities of seeds, grape seed oil and grape seed powder.

2.3 Grape seed oil and grape seed powder analysis

Determination of the regulated physicochemical quality parameters (free acidity, peroxide value, ultraviolet absorbance coefficients, fat, moisture, ashes, crude fiber, K270, K232) was carried out following the analytical methods for olive oil described by Regulation EEC 1989/2003 of the Commission of the European Union.

Analysis of acidity: Acidity, % of oleic acid, was determined by titration of a solution of oil dissolved in ethanol/ether (1:1) using 0.1 M potassium hydroxide ethanolic solution [15].

Analysis of peroxide value: Peroxide value (meq/kg) was determined using a mixture of oil and chloroform/acetic acid that was left to react with a solution of potassium iodide in the dark for five minutes. The free iodine was titrated with a sodium thiosulfate solution [15].

Determination of K270 and K232: K270 and K232 were calculated from the absorption at 270 and 232nm using a double beam spectrophotometer (AnalytikJena Specord 200) using a 1% solution of oil in cyclohexane and a path length of 1cm.

Moisture and ash analysis: Each grape seed powder sample was homogenized and analyzed for moisture (oven drying method) according to the standard AOAC procedures [1].

Crude fibre analysis: Crude fiber was determined in Selecta Digester Dosi-Fiber. Crude fiber is lost on ignition of dried residue remaining after the digestion of the sample with 1.25% (w/v) sulfuric acid (H_2SO_4) and 1.25% (w/v) NaOH solutions under specific conditions [2].

Fat value analysis: Fat was determined in grape seeds and in grape seed powder by Soxhlet extraction system using hexane as solvent for 6 hours [17].

Absorption spectroscopy in 400-800nm: Absorption spectroscopy was performed across the spectrum 400-800nm with spectrophotometer.

3. Results and discussion

Sample	Acidity (%)	Peroxide index (meq/kg)	Moisture %	Ashes %	% Crude fiber	K 270	K 232
SAV.	1,54	70	7,02	3,2	48,8	1,10	3,30
ASY.	0,06	97,5	7,0	3,15	46	0,52	3,45
MAVR.	0,08	50	5,76	4,01	47,29	0,83	3,82
MALAG	0,14	100	5,8	2,82	48,03	0,87	3,69
ROD.	0,28	152,5	5,6	2,89	47,93	1,06	3,60

Table 2. The results obtained from the measurements of grape seed oil and grape seed powder.

Acidity of grape seed oil

Examining the acidity of the samples, it was found that sample 1 (Savatiano Domaine Matsa) had the higher consumption (54,5ml) with a difference from the other samples. This may have happened due to the high pressure during the extraction, fact that is also showed from the color of the oil and the amount of oil residue. If we can compare grape seed oil to olive oil, the grape seed oil from Malagouzia grape can be classified as virgin olive oil (0.8-2%), whereas the others (acidity 0,05%-0,2%) can be classified in the category of extra virgin olive oil.

Peroxide value of grape seed oil

Peroxide value is a criterion of oxidative status for the oils.

In fresh olive oil the peroxide value is usually less than 10 meqO₂ / kg [15]. Regarding grape seed oil, samples range for peroxides are 50-152,5 meqO₂ / kg, showing that the samples have been subjected to chemical oxidation due to the effects of light, heat, the presence of oxygen and metal components possibly due to contact when pressed with a hydraulic press.

An interesting point to be noted is the number of peroxides derived from the unique red variety of the samples of grape seed oil. More specifically, in Mavroudi Arachovis the peroxide value was 50 meqO₂ / kg, which is the minimum value of all samples. This component can be combined with the fact that white wines are more sensible to oxidation while red ones show less sensitivity; therefore it may also be applied in grape seed oils from red and white grape varieties. The above result could be confirmed by further research, comparing grape seed oil from red and white varieties.

Moisture and ashes in grape seed powder

The moisture in grape seed powder samples ranges from 5.6% to 7%. Compared with wheat flour moisture content (11%-14% depending on the origin) is much less, which is justified by the drying that has occurred during the processing of seeds. The moisture content of grape seed powder tends to approach that of the roast coffee (5%).

The ashes in grape seed powder samples ranges from 2.8% to 4% and is within the limits given for food (<5%), but much more than the limits provided for wheat flour and whole wheat flour (1.6%).

The relatively high ash content of grape seed powder is a positive parameter, since it may possibly diminish the growth of certain microorganisms.

Crude fiber in grape seed powder

Crude fiber in grape seed powder samples was 46%-48%, much higher than 7% which was found in the bran sample.

The above point is a particularly important feature for grape seed powder since it can be used as an ingredient in human food, taking into account the importance of fiber in human diet.

Fat determination in seeds and grape seeds powder

Sample	% quantity of grape seed oil (Soxhlet-Grape seeds)	% quantity of grape seed oil (Soxhlet-Grape seed flour)	% quantity of grape seed oil (mech. extraction)
SAV.	10,72	7,7	4,6
ASYR.	9,72	7,46	5
MAVR.	8,324	8,22	8
MALAG.	10,48	6,87	5,8
ROD.	8,402	8,58	7,7

Table 3. % quantity of grape seed oil from seeds and powder extracted by Soxhlet and mechanical extraction.

The determination of % fat found in seeds was from 8.3% to 10.72% while in grape seed powder from 6.8% to 8.5%.

The seeds clearly contained more amount of fat relative to grape seed powder, as the second's oil had already been extracted. Nevertheless, the differences between them are much less than expected.

Grape seed oil values obtained from the seeds via Soxhlet method and via mechanical pressure differ around 5%. By using Soxhlet method we obtained greater quantity of grape seed oil relative to the mechanical method. Therefore, the usage of mechanical extraction produces less quantity of grape seed oil but the extracted grape seed oil has better quality since no solvent is used while extracting it.

K232, K270 values

In grape seed oil, K232 values ranged from 3.30 up to 3.82, indicating the advanced oxidation of the samples and K270 values vary from 0.5 up to 1.1, which is very high and involves oxidation and deterioration of the product due to exposure in high temperatures.

Absorbance in spectrum 400-800nm

All five samples exhibit maximum absorption near 400nm. Two of the samples, the Malagouzia and Roditis exhibit their maximum absorption near 600nm

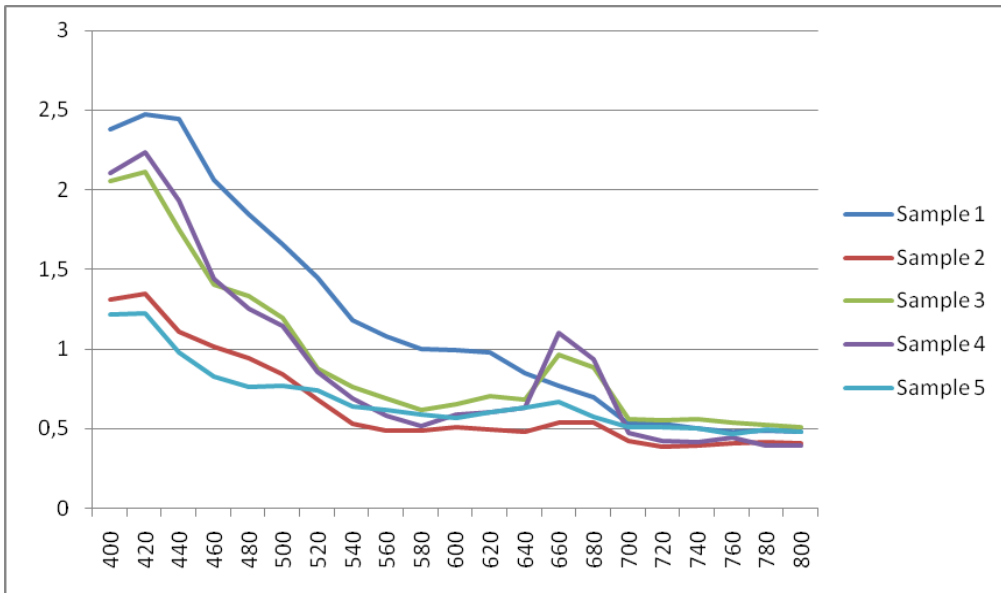


Table 4. Absorbance in 400-800nm for the samples

4. Conclusions

Grape seed oil, produced mechanically is considered to be an advantage for the quality of the product and for that reason; it can be used in food industry. Moreover, grape seed powder, also, offers a quantity of grape seed oil but it can be valuable as it is rich in crude fibers.

The present study demonstrated that grape seed oil and grape seed powder have compounds with beneficial health effect, allowing the valorization of winery by products that are not widely valorized in Greece.

References

1. AOAC Official methods of analysis of the association of official analytical chemists. Washington, USA. Association of Official Chemists (1990)
2. AOAC official method 962.09. :Fiber (crude) in animal feed and pet food W. Horwitz (Ed.), Official Methods of Analysis of AOAC International, Section 4.6.01, AOAC International, Gaithersburg, MD (2000)
3. Aparicio R., Roda L., Albi M.A., Gutierrez F. Effect of various compounds on virgin olive oil stability measured by Rancimat. *J. Agric. Food Chem.* 47. 4150-4155 (1999)
4. Arienzo, M., Christen, E.W., Quayle W.C.: Phytotoxicity testing of winery wastewater for constructed wetland treatment. *Journal of Hazardous Materials*, 169(1-3), 94-99 (2009)
5. Axtell, B.L.: Minor oil crops FAO agricultural services bulletin 94 (1992)
6. Bail.S., Stuebiger. G., Unterweger. H., & Buchbauer. G.: Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols. *Food Chemistry*. 108. 1122-1132 (2008)
7. Baydar. N., Akkupt., M.: Oil Content and Oil Quality Properties of Some Grape Seeds. *Turk J Agric.* 163-168 (2001)
8. Baydar. N., Özkan G., Cetin S.: Characterisation of grape seed and pomace extracts. *Gracas y Aceites*. 29-33 (2007)
9. Codex Alimentarius Commission Codex standard for named vegetable oils. Codex stan 210-1999 (1999)
10. Conde, E., Moure, A., Domínguez H., .Parajó, J.C. :Production of antioxidants by non isothermal autohydrolysis of lignocellulosic wastes. *LWT-Food Science and Technology*, 44(2), 436-442 (2011)
11. Conti A., Rogers, J., Verdejo, P., Harding, L.R., Rawlings.A.V.: Seasonal influences on stratum corneum ceramide 1 fatty acids and the influence of topical essential fatty acids. *J Cosmet Sci.*;18: 1-12 (1995)
12. Demirin. Y.: Phenotypic Variability and Correlation between Tocopherol Content and some Biochemical Characters in Sunflower Seeds. *Sci. Tech Bull. VKIIMK. Krasnodar.* 93:21-24 (1986)
13. Díaz, A.B., De Ong, I., Caro, I., Blandino, A. : Production of hydrolytic enzymes from grape pomace and orange peels mixed substrate fermentation by *Aspergillus awamori*. *Chemical Engineering Transactions*, 17, 1143-1148 (2009)
14. Duba, K. S, Fiori, L.: Supercritical CO₂ extraction of grape seed oil: Effect of process parameters on the extraction kinetics. *The Journal of Supercritical Fluids*. 98, 33-43 (2015)
15. EC 1991 Commission Regulation (EEC) 2568/91 of July 11th 1991 on characteristics of olive oil and on the relevant methods of analysis. Official EC J., L248, 0001-0083.
16. FAOSTAT. <http://faostat.fao.org> (2010)
17. Fernández CM, Ramos MJ, Pérez A, Rodríguez JF.: Production of biodiesel from winery waste: extraction, refining and transesterification of grape seed oil. *Bioresour Technol.* 101(18):7030-5 (2010)
18. Fiori, L.: Supercritical extraction of grape seed oil at industrial-scale: Plant and process design, modeling, economic feasibility. *Chemical Engineering and Processing: Process Intensification*. 49,8. 866-872 (2010)
19. Freitas L., Jacques R., Richter M.F., Loviane da Silva A., Caramao E.B.: Pressurized liquid extraction of vitamin E from Brazilian grape seed oil. *Journal of Chromatography A*. 1200 80-83 (2008)
20. García-Martínez, S., Grau, A., Agulló, E., Bustamante, M.A., Paredes, C., Moral, R., Ruiz, J.J.: Use of composts derived from winery wastes in tomato crop. *Communications in Soil Science and Plant Analysis*, 40(1-6), 445-452 . (2009)
21. Gliszczynska-Swiglo. A., Sikorska. E.: Simple reversed-phase liquid chromatography method for determination of tocopherols in edible plant oils. *J. Chromatogr. A*. 1048, 195-198 (2004)
22. Grismer, M.E., Carr M.A., Shepherd, H.L. :Evaluation of constructed wetland treatment performance for winery wastewater. *Water Environment Research*, 75(5), 412-421 (2003)
23. Härtel, B.: Essential Fatty Acids and Elicosanoids in the skin: Biosynthesis, Biological and Cosmetic importance. *SÖFW-Journal.*; 124: 889-900 (1998)
24. Hojerova, J., Vinohrad 41 (6), 62003
25. Israilides, J.C., A. Smith, B. Scanlon and C.: Barnett Pullulan from agroindustrial wastes. *Biotechnology and Genetic Engineering Reviews*. Vol 16, pp. 309-324 (1999)
26. Jiang Y., Simonsen J., Zhao, Y. :Compression-molded biocomposite boards from red and white wine grape pomaces. *Journal of Applied Polymer Science*, 119(5), 2834-2846 (2011)
27. Karvela ,E.: Polyphenolic ingredients in winemaking by products: Recovery and evaluation as additives in food models. PhD thesis, Xarokopio University (2011)
28. Khanna S., Venojarvi M., Roy S., Sharma N., Trikha P. , Bagchi D., Bagchi M., SEN C. K.: Dermal Wound Healing Properties Of Redox -active Grape Seed Proanthocyanidins. *Free Radical Biology & Medicine*, Vol. 33, No. 8, 1089-1096 (2002)
29. Kurki A., Bachmann J.: Oilseed Processing for Small Scale Producers. *ATTRA* (2006)
30. Luque-Rodríguez J.M., Luque de Castro M.D., Pérez-Juan P.: Extraction of fatty acids from grape seed by superheated hexane. *Talanta* 68 126-130 (2005)
31. Manios, T., Maniadaakis, K., Boutzakakis, P., Naziridis, Y., Lasaridi, K., Markakis, G., Stentiford, E.I. :Methane and carbon dioxide emission in a two-phase olive oil mill sludge windrow pile during composting. *Waste Management*. 27(9), 1092-1098 (2007)
32. Moldes, A.B., Bustos, G., Torrado, A., Domínguez, J.M.: Comparison between different hydrolysis processes of vine-trimming waste to obtain hemicellulosic sugars for further lactic acid conversion. *Applied Biochemistry and Biotechnology*. 143(3), 244-256 (2007)
33. Moldes, A.B., Vázquez, M., Domínguez, J.M., Díaz-Fierros, F., Barral, M.T. : Negative effect of discharging vinification lees on soils. *Bioresource Technology*. 99(13), 5991-5996 (2008)

34. ÖzvurL E.B, Vural H.: Grape seed flour is a viable ingredient to improve the nutritional profile and reduce lipid oxidation of frankfurters. *Meat Science* 88.179-183 2011
35. Paradelo, R., Moldes, A.B., Barral, M.T.: Utilization of a factorial design to study the composting of hydrolyzed grape marc and vinification lees. *Journal of Agricultural and Food Chemistry*, 58(5), 3085–3089 (2010)
36. Pardo J.E., Fernández, Rubio M., Alvarruiz A.,Alonso G.L.: Characterization of grape seed oil from different grape varieties (*Vitis vinifera*). *Eur. J.Lipid Sci. Techol.*, 111,188-193 (2009).
37. Pardo, A., Perona, M.A., Pardo, J.:Indoor composting of vine by-products to produce substrates for mushroom cultivation. *Spanish Journal of Agricultural Research*. 5(3), 417–424 (2007)
38. Perde-Schrepler M., Chereches G., Brie I. , Tatomir C., Postescu I.D.,Soran L, Filip A.: Grape seed extract as photochemopreventive agent against UVB-induced skin cancer. *Journal of Photochemistry and Photobiology B:Biological* 118 16-21 (2013)
39. Ping, L., Brousse, N., Chrusciel, L., Navarrete, P., Pizzi, A.: Extraction of condensed tannins from grape marc pomace for use as wood adhesives. *Industrial Crop and Products*, 33(1), 253–257 (2011)
40. Portilla, M., Torrado-Agrasar, A., Carballo, J., Domínguez J.M., Moldes, A.B.: Development of a factorial design to study the effect of the major hemicellulosic sugars on the production of surface-active compounds by *L. pentosus*. *Journal of Agricultural and Food Chemistry*, 57(19), 9057–9062 (2009)
41. Portilla, O., Torrado, A., Domínguez, J.M., Moldes, A.B.: Stability and emulsifying capacity of biosurfactants obtained from lignocellulosic sources using *Lactobacillus pentosus*. *Journal of Agricultural and Food Chemistry*, 56(17), 8074–8080 (2008)
42. Portilla, O., Torrado, A.M., Domínguez J.M., Moldes, A.B. : Stabilization of kerosene/water emulsions using bioemulsifiers obtained by fermentation of hemicellulosic sugars with *Lactobacillus pentosus*. *Journal of Agricultural and Food Chemistry*, 58(18), 10162–10168 (2010)
43. Rodriguez, L., Toro, M., Vázquez, F., Correa-Daneri, M., Gouiric, S., Vallejo, M.: Bioethanol production from grape marc and sugar beet pomaces by solid state fermentation. *International Journal of Hydrogen Energy*, 35(11), 5914–5917 (2010)
44. Sant'Anna V., Christiano F.D.P., Marczak L.D.F., Tessaro I.C., Thys R.C.S. The effect of the incorporation of grape marc powder in fettuccini pasta properties. *Food Science and Technology* 58. 497-501 (2014)
45. Sharif A, Akhtar N, Khan MS, Mena A, Mena B, Khan BA, Mena F.: Formulation and evaluation on human skin of a water-in-oil emulsion containing Muscat hamburg black grape seed extract. *Int J Cosmet Sci* 37(2):253-258. doi: 10.1111/ics.12184 (2015)
46. Takahashi T. ,Kamiya T, Hasegawa A.,Yokoo Y: Procyanidin oligomers selectively and intensively promote proliferationof mouse hair epithelial cells in vitro and activate hair follicle growth in vivo. *Journal of Investigative Dermatology*. 112. 310–316 (1999)
47. Torres J.L., Varela B., Garcia M.T., Carilla J., Matito C., Centelles J.J., Cascante M., Sort X., Bobet R.:Valorization of grape (*Vitis vinifera*) byproducts. Antioxidant and biological properties of polyphenolic fractions differing in procyanidin composition and flavonol content. *J. Agric. Food Chem.* 50, 7548-7555 (2002)
48. Vatai, T., Skerget M., Knez, Z. :Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide. *Journal of Food Engineering*. 90(2), 246–254.dioxide. *Journal of Food Engineering*. 90(2), 246–254 (2009)
49. Yousafi. M., L.Nataghi., Gholamian., M.: Physicochemical properties of two type of shahrodi grape seed oil (Lal and Khalili). *European Journal of Experimental Biology*, 3 (5):115-118 (2013)