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Phytochemical Investigation and Radical Scavenging Activity of Wastes of Some Grape Varieties Grown in Egypt

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Abstract: The skins and seeds of some grapes wastes grown in Egypt were screened for the presence of major phytochemical groups. Phytochemical screening of grape wastes alcoholic extracts showed the presence of flavonoids, carbohydrates, tannins, triterpenoids, steroids, as major groups, while alkaloids were absent in all grape wastes. Qualitative and quantitative analyses of lipoidal content of the different extracts from different cultivars (Red Roomy, Thompson seedless, Crimson seedless, Grenache noir) of *Vitis vinifera* L. grown in Egypt were performed. In addition, the antioxidant contents of grape seed oil and pomace oil of these wastes were determined. Oil and/or lipoidal matter in dried, powdered seed and pomace materials were extracted with pet Ether. Oil concentration of seeds ranged from 11.8 to 12 % while in pomace the oil varied from 3.1 to 9.5%. Grape seed and pomace oils were rich in oleic and linoleic acids and the degree of unsaturation in the oils was over 70%. Alpha-tocopherol was the most abundant tocopherol in the oil, while δ-tocopherols were higher in Thompson seedless and lower in Crimson seedless, Red roomy skin and seeds

Key words: Antioxidant activity · Grape waste · Lipoidal matter · Phytochemical screening · Tocopherol

INTRODUCTION

Recycling of agriculture and industrial residues is one suitable technology adopted in many countries. In the last few years, an increased attention has been focused on the industrial wastes, especially those containing residual phenols from wastes. Grapes (*Vitis vinifera*) are the world's largest fruit crop with more than 60 million tons produced annually. About 80% of the total crop is used in wine making [1] and pomace represents approximately 20% of the weight of grapes processed. In Egypt, grapes are considered the second important crop after citrus. Grapes growing area is about 152.488 faddan (one faddan=0.42ha) producing about 200.000 ton fruits. Pomace represents about 10 to 20 thousand tons/year [2]. Grape pomace is characterized by high-phenolic contents because of poor extraction during wine industry, making their utilization worthwhile and thus supporting sustainable agricultural production [3]. In recent years, the use of grape seed extracts (GSE) has gained ground as a nutritional supplement in view of its antioxidant activity [4]. The by-products obtained after winery exploitation, either seeds or pomaces, constitute a very cheap source for the extraction of antioxidant flavanols, which can be used as dietary supplements, or in the production of phytochemicals, providing an important economic advantage [4-6] adding additional value to the residue.

Grape seed contains around 13% of oil with high level of linoleic and oleic acids. Bail *et al.* [7] reported a total polyphenol content ranging from 59 to 115.5 mg/g as gallic acid in grape seeds. Grape seed oil contains 399.785 mg/kg vitamin (E) depending on variety and environmental conditions [8]. Phenolic substances or polyphenols include many classes of compounds ranging from phenolic acids i.e. cinnamic, coumaric, caffeic, ferulic,

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chlorogenic and gallic acid, to flavonoids including colorless flavan-3-ols such as catechin, epicatechin and their polymers and ester forms. Also, flavanones, quercetin and red and blue anthocyanins [9, 10]. Polyphenols can be considered as added value by-products, justifying their isolation from industrial wastes [11]. These residues in fact could be an alternative source for obtaining natural antioxidants which are considered completely safe in comparison with synthetic ones such as butylated-hydroxy anisole (BHA) and butylated hydroxyl toluene (BHT) compounds. These synthetic compounds are largely used in food industry with undesirable effects on the enzymes of human organs [12]. In terms of pharmacological activity, polyphenols protect against the oxidation of high density lipoprotein HDL. Additionally, they have anti-ulcer, anticarcinogenic and antimutagenic activities. These strong activities are due to their ability to scavenge the free radicals [13]. For this reason, the objectives of this study are the exploitation of by-products from wine making industries in Egypt and investigate the feasibility of extracting high-value phytochemicals from common grape varieties either seedless or seeded ones grown in Egypt.

MATERIALS AND METHODS

Grape Pomace Samples: Four grape varieties were selected for this study and their wastes were produced and obtained as follows:

- Grenache noir waste obtained from El Kroom Company, consist of skin and seeds (red berries).
- Thompson seedless waste obtained from El Kroom company, Alexandria, Egypt, which consist of skin only as the species is seedless one (white berries).
- Red roomy consists of skin and seeds, obtained from local market and pomace was produced in the Medicinal and Aromatic Plants Department, NRC (red berries).
- Crimson seedless grape obtained from local market and pomace produced in the lab (red berries).

Each sample was hand divided into their parts; skin, seeds and pomace. Seeds obtained were air dried and weighted, pomace was oven dried at 50°C, then ground to a fine powder. The powdered samples were then kept at -4°C until used.

Preliminary Phytochemical Screening: Absolute ethanol extracts obtained were subjected to preliminary phytochemical screening and the following tests were done to check the presence of phytoconstituents. Test for Alkaloids (Dragendorff test [14]) Flavonoids (Alkaline reagent test [15]), Carbohydrates (Molischs test [16]), Saponins (Forth test[17]), Tannins[17], Triterpenoid (Libermann Burchard test [18]) and coumarins as described by Feigl [19].

Determination of Total Lipid Contents: Accurate weight (2g) of air-dried fine powder of the grape wastes were extracted by petroleum ether 40-60°C using Soxhlet apparatus till complete extraction. The solvent was evaporated by rotary evaporator at 40°C till dryness then the residue was kept in vacuum desiccators till constant weight and the percentage of total lipids was calculated.

Total lipids % = weight of the extract x100/ weight of waste material

Identification of Unsaponifiable Matter and Fatty Acids of Different Grape Wastes:

Separation of Unsaponifiable Matter and Fatty Acids: An aliquot of the oil (1ml) was saponified with methanolic KOH (20ml, 10%) at 80°C for 3h under reflux. The unsaponifiable matter was extracted with ether (10 x 10 ml), washed several times with distilled water, dried over anhydrous sodium sulphate. Then the solvent was evaporated and the unsaponifiable matter was weighed and kept for further analysis. The soap solution was acidified with HCl (10%), the liberated fatty acids were extracted with ether (3 x 30 ml), washed several times with distilled water till acid free, dried over anhydrous sodium sulphate. The solvent was evaporated and the fatty acids were weighed [20].

Identification of the Unsaponifiable Matter: The unsaponifiable matter of grape wastes was identified by using GLC (Central Services Lab. NRC), with the following conditions: Hewlett Packard HP 6890 apparatus equipped with HP-1 methyl siloxane capillary column (0.25 mm x 30 m), using flame ionization detector (FID), nitrogen was used as a carrier gas, Nitrogen; hydrogen and air gases were set at flow rates 30, 30 and 300 ml/min, respectively. Oven temperature was programmed from 70-280°C at a rate 8°C/min. Temperatures of detector and injectors were 300 and 250°C, respectively. The hydrocarbon and sterol compounds were identified by comparing the relative retention times of the separated components with those of available standard materials injected under the same conditions. The quantitative estimation of each compound was based on the area of the recorded peak area.

Preparation of Fatty Acid Methyl Esters: Methyl esters of fatty acids were prepared by refluxing 10 mg of the liberated fatty acids with 10 ml (2%) of H_2SO_4 in anhydrous methanol for 5 hr on a water bath at 90°C [21]. The fatty acid methyl esters were extracted with pet. ether (10 ml/each). The pet. ether extract was treated with diluted sodium bicarbonate solution to remove the acidity, washed several times with distilled water, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure.

Identification and Quantitative Determination of Fatty Acids by GLC: The fatty acid methyl esters were identified using GLC stand in Central Services Lab. NRC under the following conditions: Pye Unicam PU 4550 apparatus equipped with a coiled glass column (4mm x 1.5m, i.d), packed with diatomite-C (100-120 mesh) and coated with 10% polyethylene glycol adipate "PEGA", with flame ionization detector (FID). Nitrogen was used as a carrier gas, hydrogen and air gases flow rates were at 30, 33 and 330 ml/min, respectively. Oven temperature was programmed from 70 to 190°C, increased by 10°C/min. Temperatures of detector and injector were 300 and 250°C, respectively. Fatty acids were identified by comparing the relative retention time of each peak with those of standard fatty acid methyl esters injected under the same conditions.

Tocopherol Analysis: Tocopherol composition of the samples was determined as described by Turan *et al.* [22]. Tocopherols were analyzed by HPLC (*ZORBAX* Eclipse *XDB* C18 column). The normal phase column in the system was an Inertsil NH_2 column (250 mm x 4.6 mm, 5ìm) and the column temperature was maintained at 30°C. Separation of tocopherols was based on isocratic elution with *n*-hexane (96%) and isopropanol (4%) at 1 ml/min. The eluate was monitored at 292 nm by using a photodiode-array detector (SPD-M20A). The compounds were identified by comparing their retention times and the UV spectra with the authentic standards. Tocopherols were quantified based on the peak areas compared with an external standard. Tocopherol analysis was performed in triplicate for the single samples of each variety and the average values were calculated.

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) Radical Scavenging Activity: The determination of ABTS was performed according to procedure described by Muller [23] with slight modifications. In a typical experiment, 0.1ml of potassium phosphate buffer (0.1 M, pH 5.0), was mixed with 20 µl of hydrogen peroxide (10 mM) and 0.1ml waste extracts (100 µl/ml). The mixture was then incubated at 37°C for 5 min. A mixture of 30 µl ABTS (1.25 mM, in 0.05 M phosphate-citrate buffer, pH 5.0) and 30 µl peroxidase (1 unit/ml) were then added and further incubated at 37°C for another 10 min. The absorbance of the mixture (AA) was measured at 405 nm against a blank (AB) on a multiplate reader (Tecan Austria) using alpha-tocopherol as positive control. The ABTS radical scavenging activity was measured from the following equation:

$$\text{ABTS radical scavenging activity (\%)} = [1-(AA/AB)] \times 100$$

RESULTS AND DISCUSSION

Chemical characterization of winery waste was a prior necessity in order to evaluate its potential, to determine the extraction yield and to be controlled qualitatively.

Qualitative Phytochemicals Screening: The phytochemical screening of grape seed and skin showed the presence of flavonoids, carbohydrates, tannins, triterpenoids, steroids, while alkaloids, saponin, coumarins and volatile substances were absent in all grape wastes.

Oil Content and Identification of Lipoidal Matter of Different Grape Wastes: Appreciable amounts of seeds could result as a waste from manufacturing. Consequently, a considerable amount of edible oil could be obtained. GÖktürk and Akkurt [24] concluded that grape seed oil could be considered as an important source for production of edible oil. Grape seed oil represents a promising plant fat, which is composed of average 90% poly and monounsaturated fatty acids, responsible for its value as nutritive edible oil. Furthermore an unusual high smoke point (about 190-230°C) has been reported for grape seed oil, making it suitable for cooking at high temperature [25]. The oil contents of grape seeds and pomace extracted from four different wine-grape cultivars are given in Table 2. The oil contents of grape seeds ranged from 11.8% (Grenache noir seeds) to 12% (Red roomy seeds). Oil content of grape strongly depends on grape variety though the usual range is 5-16% of dry weight [24]. These results are in good agreement with those obtained by Göktürk and Akkurt [23] and Ohnishi *et al.* [26]. They reported that the oil content of seeds obtained from different grape cultivars ranged from 9.90 to 20.00%.

Table 1: Phytochemical screening of grape waste extracts.

Test	Grenache noir waste	Grenache noir seeds	Red roomy skin	Red roomy seeds	Crimson seedless skin	Thompson seedless skin
Terpenoids/ volatile substances	-	-	-	-	-	-
Carbohydrate or glycosidea	++	++	++	++	++	++
Tannins	+	++	+	++	+	+
Flavonoids, NaOH	+	++	+	++	+	+
Flavonoids, Shinoda	+	++	+	++	+	+
Saponin	-	-	-	-	-	-
Sterol and / or triterpenes	+	+	+	+	+	+
Coumarins	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-

(++), (+) and (-) refer to high, low and absent amount, respectively.

Table 2: Total lipids content (%) and relative percentage of unsaponifiable and saponifiable compounds of different wastes.

Content	Grenache noir seeds	Grenache noir skin	Red roomy seeds	Red roomy skin	Crimson seedless wastes	Thompson seedless skin
Crude oil	11.8	9.5	12	3.1	1.65	6.4
Unsaponifiable matter	20	20	19	28	28	29
Saponifiable matter	77	70	80	63	62	66

Table 3: GLC analysis of unsaponifiable matter of different wastes.

			Relative percentage					
Unsaponifiable matter	No. Carbon atom	RRt*	Grenache noir seeds	Red roomy seeds	Grenache noir skin	Crimson seedless skin	Red roomy skin	Thompson seedless skin
Tetradecane	C14	0.61	6.89	4.28	0.23	-	2.48	0.28
Pentadecane	C15	0.72	-	2.63	0.36	-	0.62	-
Hexadecane	C16	0.79	0.003	0.72	1.37	-	0.73	0.47
Heptadecane	C17	0.83	3.05	2.08	1.69	-	-	0.71
Octadecane	C18	0.92	0.003	0.93	5.79	3.31	2.54	0.91
Nonadecane	C19	0.98	0.75	-	4.26	-	-	0.53
Eicosane	C20	1.00	1.12	5.63	5.44	1.04	2.68	2.04
Heneicosane	C21	1.04	18.5	20.2	0.003	7.02	2.48	3.98
Docosane	C22	1.13	1.22	6.08	3.57	0.78	1.17	1.58
Tricosane	C23	1.15	12.10	-	6.68	2.56	4.70	4.21
Tetracosane	C24	1.20	3.31	1.05	7.52	4.65	5.38	8.66
Pentacosane	C25	1.23	9.68	2.03	4.58	2.30	-	-
Hexacosane	C26	1.25	8.36	2.64	4.24	4.36	6.7	9.14
Heptacosane	C27	1.36	6.32	4.34	7.44	0.48	3.12	9.45
Octacosane	C28	1.39	7.03	5.52	3.57	19.3	7.45	24.9
Nonacosane	C29	1.47	3.42	-	2.82	10.8	6.69	-
Triocontane	C30	1.52	6.32	3.49	7.76	18.2	8.85	7.48
Cholesterol	--	1.61	-	-	-	-	-	-
Campasterol	--	1.63	-	-	5.03	-	20.0	11.20
Stigmasterol	--	1.66	3.69	31.8	10.10	6.14	-	14.50
β-Sitosterol	--	1.72	8.19	6.61	17.50	19.00	24.4	0.28

*Rrt: Relative retention time according to retention time of eicosane 20.93 min.

Identification of Unsaponifiable Matter and Fatty Acids of Different Grape Wastes: Analysis of Unsaponifiable Matter: The data of GLC analysis for unsaponifiable matter of different grape wastes are shown in Table 3 . A total of 21, 18, 17, 17, 16, 14 sterols or hydrocarbones were identified for the lipoidal matter of Grenache noir seeds, Thompson seedless, Red roomy skin, Red roomy seeds and Crimson seedless skin were obtained, respectively. The results revealed the presence of hydrocarbons ranging from C_{14} to C_{30} of which tricosane, heptadecane, dotriacontane, eicosane and hexadecane were the most predominant components. Dealing with sterols, the unsaponifiable matter of different grape wastes contained campesterol, stigmasterol and β-sitosterol.

Analysis of Fatty Acids: The results in Table 4 show the fatty acid methyl esters of different grape wastes under investigation. Gas-liquid chromatography was used in this investigation for the qualitative and quantitative determination of individual fatty acid. A total of (18, 14, 13, 11, 11, 8 fatty acids) were identified in the fatty acid methyl esters of the lipoidal matter of Red roomy seeds, Grenache noir seeds, Grenache noir skin, Red roomy skin, Crimson seedless skin and Thompson seedless, respectively (Table 5). In Grenache noir wastes, Red roomy skin and Crimson seedless skin, the saturated fatty acids represent 28%, 26% and 29% of which palmitic acid was identified as major saturated fatty acid (7.55, 9.85 and 22.5, respectively) (Table 5). Whereas, Grenache noir skin contain about (33%) saturated fatty acids of which lauric is a major (4.2%), besides many other saturated fatty acids were present in traces. In Red roomy seeds, the major saturated FA was heptadecanoic (15%) while the major saturated FA in white wastes was arachidic (16%) of which total saturated fatty acids represent 33 and 25%, respectively. The total fatty acid fraction of grape wastes under study contained high percentage of unsaturated fatty acids, while saturated fatty acids were present as minor. The major component in the fatty acid methyl esters of Red roomy seeds (38%) was identified as Cis 13,16-Decosadenoic (22:2), followed by oleic acid (23%) while the major component of the fatty acid methyl esters of GN seeds was identified as lenoleic acid (43%), the major component in the fatty acid methyl esters of Grenache noir wastes was identified as lenoleic acid (47%) followed by Cis 13,16-Decosadenoic (22:2) (10.7%). On the other hand the major fatty acid methyl esters in Crimson seedless skin and Thompson seedless waste was lenoleic acid (58.5 and 68, respectively) and the major in Red roomy skin was myristoleic (26.3%) followed by lenoleic acid (17.1) (Table 5). The percentage of major unsaturated fatty acid (linoleic) in all the wastes studied are as follows: 68, 58.5, 43, 47, in Thompson seedless, Crimson seedless, Grenache noir skin and Grenache noir, respectively, however oleic acid was found to be major in Red roomy skin and seeds 23.4 and 23 % (Table 4). The major unsaturated fatty acid in all grape seed and pomace under study was linoleic acid as follow; 68% in Thompson seedless, 58.5% in Crimson skin, while oleic acid amounted to 23.4 and 23.0% in Red Roomy skin and seeds, respectively (Table 5). In the present investigation, among the identified fatty acids, linoleic acid (C18:2) was predominant. In the second order came oleic acid (C18:1). These are in agreement with those determined in grape seed oil by Rodríguez *et al.* [27] and Tangolar *et al.*[28]. The degree of unsaturation in the grape seed oil (Table 4) was over 86%, coming from unsaturated fatty acids. High levels of unsaturation play an important role in lowering high blood cholesterol and also in the treatment of atherosclerosis [29].

Poly-unsaturated fatty acids such as linoleic and linolenic are essential fatty acids for the human body because they cannot be synthesized in the body. From this point of view, grape seed oil, which is rich in linoleic acid and poor in other acids, may be a valuable source of dietary fat. Low levels of linolenic acid are preferred in edible oils, because high levels of this fatty acid can cause unfavorable odor and taste. Furthermore, since 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. Due to these facts; low quantity of linolenic acid and its short shelf-life, grape seed oil has important advantage in terms of human health. The second abundant fatty acid in seed and pomace oil was oleic acid. It is, a monounsaturated fatty acid, has great importance in terms of their nutritional implication affect the oxidative stability of oils [30]. Saturated fatty acid values were less than the values of monosaturated ones and also polyunsaturated fatty acids in grape seed oil. The ratio of MUFA, PUFA and SFA of grape seed oil were 84%, 53.01 % and 16.7%, respectively. Two types of PUFA n-6 (linoleic) and n-3 (linolenic), are important with regard to health and stability of grape seeds [31]. The present results are similar to those found by Göktürk and Akkurt [24], Beveridge *et al.* [32] and Barron *et al*. [33]. They reported that in pomace oils, the following acids were recorded in the following percentages. 8.60 to 10.63%, 3.58 to 4.59%, 16.07 to 22.57%, 61.16 to 69.97% and 0.47 to 0.63% for palmitic, stearic, oleic, linoleic and linolenic acids, respectively. The fatty acid composition of grape seed and pomace are similar to the oils of safflower, sunflower, soybean, maize, cotton seed, poppy and tobacco, which belong to the linoleic type.

Tocopherol Contents: Alpha tocopherol contents of grape seed and pomace oil extracted from grape cultivars are presented in Table 6. Alpha-tocopherol was the most abundant tocopherol in the oil, however δ-tocopherols were found in Thompson seedless and with low concentration in Crimson seedless, Red roomy skin and seeds. Similarly, Baydar *et al.* [34] reported that oil extracts from Thompson seedless pomace gave the highest tocopherol contents compared to the seeds. The pomace oils of Thompson seedless exhibited the highest α-tocopherol values as compared to the other

Table 4: Percentage of unsaturated and saturated fatty acids of different wastes.

	Relative percentage					
Fatty acids	Red roomy seeds	Grenache noir seeds	Grenache noir skin	Crimson seedless skin	Red roomy skin	Thompson seedless skin
Monounsaturated	23	2	4	1	50	6
Polyunsaturated	45	65	68	70	24	69
Unsaturated	68	67	72	71	74	75
Saturated	32	33	28	29	26	25

Table 5: GLC analysis of fatty acid methyl esters of different grape wastes.

			Relative percentage					
Fatty acid	No. carbon atom	*RRt	Red roomy seed	Grenache noir seed	Grenache noir skin	Crimson seedless skin	Red roomy skin	Thompson seedless skin
Butryic	(C4:0)	0.50	3.0	-	0.3	0.08	0.51	-
Caproic	(C6:0)	0.58	1.2	-	-	-	-	-
Caprylic	(C8:0)	0.65	0.8	0.3	-	-	-	-
Capric	(C10:0)	0.72	1.6	-	-	0.17	-	-
Undecanoic	(C11:0)	0.76	0.4	0.2	-	-	0.97	-
Lauric	(C12:0)	0.79	3.0	4.2	1.11	-	-	-
Myristic	(C14:0)	0.92	4.2	10.0	2.11	0.45	-	1.4
Myristoleic	(C14:1)	0.93	0.3	0.5	1.09	0.18	26.3	1.9
Palmitic	(C16:0)	1.00	6.5	1.7	7.55	22.5	9.85	4.1
Heptadecanoic	(C17:0)	1.04	5.1	15.0	4.42	3.34	9.48	4.3
Stearic	(C18:0)	1.07	0.2	0.6	2.13	-	-	-
Oleic	(C18:1)	1.09	23.0	1.1	3.15	0.53	23.4	3.9
Lenoleic	(C18:2)	1.14	3.5	43.0	47.1	58.5	2.16	68
Lenolenic	(C18:3)	1.19	3.6	14	7.1	8.28	17.1	0.8
Arachidic	(C20:0)	1.35	1.7	-	2.98	2.46	2.75	16.0
Cis-11,14-Eicosadienoic	(C20:2)	1.47	2.5	8.1	2.94	-	-	-
Behenic	(C22:0)	1.58	1.7	1.1	7.3	-	2.54	-
Cis 13,16-Decosadenoic	(C22:2)	1.71	38	-	10.7	3.52	4.92	-

*Rrt: Relative retention time according to retention time of palmitic acid (19.87 min).

Table 6: Tocopherols Content of grape seeds and pomace.

	α (RT 6.2)	Delta (RT 5.2)
Grapes	mg/kg oil	
Red roomy skin	8	10
Red roomy seeds	12	0.6
Crimson seedless skin	1.6	0.3
Thompson seedless waste	64	25
Grenache noir waste	3	-
Grenache noir seeds	2.4	-

cultivars. Similarly Göktürk and Akkurt [24] and Aguilera *et al.* [35] reported that the tocopherol contents changed depending on grape genotype. Published studies on the tocopherol contents of grapes are mostly focused on grape seed oil [24, 36]. Gliszczynska-Swiglo and Sikorska [37] found that α, γ+ β and δ-tocopherols accounts of 100.55 mg/kg, 17.14 mg/kg and 3.89 mg/kg, respectively. Tocopherol contents varied not only from one oil source to another but also among cultivars. Thompson seedless cultivar exhibited the highest α, δ and total tocopherol values as compared to the other cultivars. Tracing literature few studies were found on the tocopherol content in grape seeds. The content of alpha-tocopherol in the present investigation is in good agreement with the results given in the literature for grape seeds. In one study it was between 0.8 mg/100 g to 2.4 mg/100g [38] and in another study it was between 20.51 mg/kg to 34.01 mg/kg [34], although the origins of the grape seeds analyzed are different. This is an expected result, since tocopherols have been concentrated in grape seed oils. Fernandes *et al.* [39] showed that the seed oils were a good source of γ-tocotrienol (499-1575 mg/kg), δ-tocopherol (85.5-244 mg/kg) and α-tocotrienol (69-319 mg/kg). These results could be a useful guide to consumers who wish to produce tocopherol-rich grapes seeds which will be a good contributor to a balanced intake of vitamin E.

Antioxidant Activity of Tocopherol and Waste Oil: Vitamin E is a generic name used to describe a group of compounds (namely tocopherols and tocotrienols) that

Table 7: *In vitro* ABTS radical scavenging activity of different grape wastes oil.

Grapes	% Inhibition (mg/100µl)	Equivalent to alpha tocopherol (ppm)
Grenache noir seeds	9.2 ±0.7	0.03
Red roomy seeds	11 ±0.7	0.04
Red roomy skin	10 ±0.7	0.03
Crimson seedless skin	14 ±0.8	0.05
Thompson seedless waste	58 ±1.1	0.3
Grenache noir	12 ±0.6	0.04

share a common structure with a chromanol head and an isoprenic side chain. They have four possible naturally occurring forms (α, γ, β and δ-tocopherols and tocotrienols) that differ in the number and the position of methyl groups attached to the chromanol head [40]. They are lipid-soluble anti-oxidants synthesized only by photosynthetic organisms and extracted as oils from plant material [41]. The biological activity of tocopherols and tocotrienols are mainly attributed to their antioxidant activity in inhibiting lipid peroxidation in biological membranes. Alpha-tocopherol is the most active form of vitamin E and accounts for almost all vitamin E activity in living tissue[42] [. The antioxidant activities of the grape oil compared to tocopherol, as determined by the scavenging of ABTS, are presented in Table 7.

Thompson seedless oil had the highest alpha-tocopherol isomer value (64 mg/kg oil), also has the highest antioxidant activity; showing correlation between tocopherol content in grape oil and their antioxidant activities. Tocopherols are important antioxidant compounds found mainly in oils. The oxidative stability of the oils is mostly based on these compounds [43]. The α-tocopherol is capable of quenching free radicals, which protects phospholipids and cholesterol against oxidation and subsequent breakdown to potentially harmful chemically reactive products [44]. Tocopherols are one of the most powerful natural fat-soluble antioxidants. It acts as antioxidants by two primary mechanisms, a chain-breaking electron donor mechanism, in which they donate their phenolic hydrogen atom mechanism, that includes singlet oxygen scavenging or quenching; this inhibits oxidations induced by electronically excited singlet oxygen [43]. Oil extracts with high tocopherol content can be used in applications where a high level of antioxidant protection is needed [45]. On the other hand, among the tocopherols present in foods, α-tocopherol shows the highest vitamin E activity, thus making it the most important for human health and biological activity [46]. Alpha-tocopherol has also been demonstrated to have beneficial effects on the oxidative and color stability of red meats, such as beef [47]. Medicinally tocopherol plays an important role in the prevention of cancer, inflammatory activities and cardiovascular disease [48]. The subject of natural anti-oxidants continues to captivate the interests of food and biomedical scientists because of the reports that diets rich in plant anti-oxidants derived from fruits and vegetables are associated with lower risks of coronary heart disease and cancer [49].

CONCLUSION

Fixed oil extracted from the seed and pomace of different grape cultivars contain a large amount of unsaturated fatty acids and tocopherols. The beneficial effects of grape seed and pomace oil extracts are due not only to their high degree of unsaturation, but also to their antioxidants components such as tocopherols which may serve as dietary sources of natural antioxidants preventing diseases and promoting human health. It can be concluded that, wine byproducts and grape juice, a large scale waste, can be utilized both to get natural antioxidants and to obtain edible vegetable oil.

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