Phenolic Compounds and Antioxidant Activity of White, Red, Black Grape Skin and White Grape Seeds

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Abstract: Grape skin and seeds are sources for phenolic compounds that contribute to the sensory characteristics and beneficial bioactive of many processed foods. Hence, the study was aimed to evaluate and characterize the phenolic composition and evaluate the antioxidant activities of three grape varieties skin (white, red and black) and white grape seeds. The results indicated that among the grape skin of the three varieties, black grape skin (BGS) contained the highest amount of total phenolic compounds (2070.02mg GAE/100g dry weight). While white grape skin (WGS) found to have the lowest phenolic contents (296.27mg GAE/100g). On the other hand, white grape seeds (WG Seeds) contained the highest content of phenolic compounds compared to the skin samples (2536.5mgGAE/100g dry weight).The phenolic composition of the grape skin and grape seeds samples were determined by HPLC. The main phenolic compound in the three grape skins was Di-OH-cinamic acid. In the contrast, the main phenolic compounds in the grape seeds were Catechin and Brocyanidin B1. Besides, all the extracts showed remarkable DPPH radical scavenging activities with EC50 values ranged from 0.26-26.91µg extract/µg DPPH. The results showed that scavenging capacity of black grape skin and grape seeds extracts increased with increasing concentration of the skin extract in the range 0 – 21.08 µg extract/µg DPPH and grape seeds extract up to 1.92 µg extract/µg DPPH. Effect of addition different concentrations of grape skin and seeds extracts on oxidative stability of sunflower oil at 100 °C by Rancimat was studied. The results indicated that at low concentration 200ppm all extracts improved the oxidative stability of sunflower oil comparing to the control. The addition of 2% WGS, RGS, BGS and WG Seeds to rats diet showed significant decrease $P<0.05$ of TC, LDL-C and TG. On the other hand, 4% (RGS, WG Seeds), 8% BGS and 2% WG Seeds showed the same effects as BHT. Feeding rats on diet containing 200ppm BHT and 4% (WGS, RGS, and BGS) showed that no significant change of HDL-C compared to the control group. Serum Glucose was increase by increasing the levels of grape skin and seeds, in the diet. Feeding rats on diet containing 8% (WGS, RGS, and BGS) and WG Seeds at different levels caused a significant increase in catalase enzyme activity compared to synthetic antioxidant. Meanwhile, Feeding rats on diets containing 4% and 8% grape skin and seeds decreased liver function more than 2% compared to the control group and synthetic antioxidant. In Conclusion grape skin and seeds had higher antioxidant activity a specifically at low concentrations. Moreover, higher concentrations lead to higher decrease of liver function more than low concentration. The high phenolic content and the considerable antioxidant activity of the grape skin and seeds could be potentially considered as sources for natural antioxidants

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1. Introduction:

Lipid peroxidation during processing and storage of food is a serious problem that the development of undesirable off-flavor, potentially toxic reaction products and lowers the nutritional value of food and loss of shelf- life (*Millard et al., 1996* and *Baydar et al*., 2007). The major strategies for preventing lipid oxidation are the use of antioxidant (*Tang et al., 2001*). Antioxidants are organic compounds that, when added to food products, especially to lipids and lipid – containing foods, can increase shelf life by reducing the process of lipid peroxidation (*Anon, 2003*).

Antioxidant can interfere with the oxidation process by reacting with free radicals in one or more of the following ways: 1)- as reducing agents, 2)-As free radical scavengers, 3)- As complexes of prooxidant metals and 4)- as singlet oxygen quenchers (*Pratt and Hudson, 1990*). Some antioxidant compounds are synthetic antioxidants and others are natural dietary constituents (*Larson, 1988*). Synthetic antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Propyl gallate (PG) and tertiary butyl-hydroquinone (TPHQ) especially BHA and BHT are widely used in lipids and food that contain lipid. Results showed their possible undesirable effects and carcinogenic effect on human health. Also, abnormal affects on enzymes systems *(Jayaprakasha et al., 2003, Bayder et al., 2007,*

Monica et al., 2007 and Sayago- Ayerdi et al., 2009). Therefore interest in natural antioxidants, that can replace synthetic ones, that causes many Side effects, is increasing (*Puupponen- Pimia et al., 2005*). Plants provide a rich source of natural antioxidants. These include tocopherol, vitamin C, carotenoids and phenolic compounds (*Harboner, 1994*).

Grape (*Vitis vinifera L.*) is the world's largest fruit crop *(Maier et al., 2009*). About 80% of the total crops are used in wine-making, yielding by-products which include grapes skins and seeds (*Valiente et al., 1995*). Also during juice making from grape, high quantities of by- products (grape pulp, seeds and skin) remain, which are used only as a feed for animals due to their fiber content (*Palma et al., 2001*).

By-products of grape juice are rich phenolic compounds including flavonoides and non-flavonoids. It is a good and cheap source of high quality polyphenolic compounds which can be used in different therapeutic procedures with the purpose of free radical neutralization in biological system (*Heim et al., 2002*, *Yilmaz and Toledo, 2004*, *Balasundran et al., 2006*, *Lafka et al., 2007* and *Makris et al., 2007)*.Some of researchers reported the grape barriers are-sources for polyphenolic compounds which used as functional food additives and procyanidin rich extracted from grape seeds and skin have antioxidant properties (*Liuis et al., 2011 and Felic et al., 2012*)

This study aimed to investigate the phenolic composition and evaluate the antioxidant activity of white, red, black grape skin and white grape seeds.

2. Materials and Methods.

Materials

White grape (*Vitis vinifera L.*) By-product (skin and seeds) were obtained from Ganklees factory "Wady El-Natroon"-Alexandria Governorate, Egypt, season 2010. Red and black grape were obtained from local market – Egypt then prepared to get their by-product. Linoleic acid, Ammonium thiocyanate, Iron (II) chloride purum anhydrous and Butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich Chemical GmbH, Riedstr.2, D. 89555 Steinem, and Germany. Folin – Ciocalteu reagent, Gallic acid monohydrate and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Company. (USA). All solvents used (ethanol and methanol) were obtained from El - Goumhouria CO. 23, El Sawah St. Cairo-Egypt. Kits of blood analysis were purchased from Biodiagnostic Company. 29 Tahreer St., Dokki, Giza, Egypt.

Methods

1- Chemical evaluation

Preparation of grape by product sample

Grape skin and seeds were air dried at 40 °C for 1hr and ground into fine powder using laboratory electric mill (Braun, model 2001 DL, Germany) then stored in the polyethylene bags in the freezer at -20°C until use (*Mohamed and Girgis, 2005*).

Moisture Content, Ash, Protein, Lipid and Crude Fibers: were determined according to *A.O.A.C (2000*). Total Sugars were determined by difference.

Identification of fatty acids by chromatographs (GLC):

The method described by *Farag et al., (1986)* was applied for determination of fatty acids by GLC. The methyl esters of fatty acids obtained from oil of samples and standard materials were analyzed with a Pye Unicom Series 304 gas chromatograph equipped with dual flam ionization detector and dual channel recorder. The separation of fatty acid methyl esters was conducted using a coiled glass column (1.5 m x 4 mm) packed with Diatomite (100 - 120 mesh) and coated with 10 % polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8ºC/min from 70ºC to 190ºC, then isothermally at 190ºC for 25 min with nitrogen at 30 ml/min.

Total phenolic contents

Total phenolics were determined spectrophotmetrically using the modified Folin–Ciocalteau colorimetric method (*Asami et al., 2003*). Briefly 5ml of distilled water, 0.5- 1.0 ml of each sample of extracts, 1.0 ml of folin ciocalteu reagent was added to a 25ml volumetric flask. The contents were mixed and allowed to stand at room temperature for 5-8 min. Then 10 ml of 7% NaCO3 solution was added to the flask. After two hours, absorbance was measured at 750 nm using spectronic 2000, spectrophotometer, Busch and lamb (USA).The results are expressed as Gallic acid equivalent on fresh weight basis, mg /100g.

Total anthocyanins

Total anthocyanins content of grape by-products samples (White Grape Skin (WGS), Read Grape Skin (RGS), Black Grape Skin (BGS) and White Grape Seed (WG Seeds)) was measured using the pH differential absorbance method described by *Worlstad and Giusti, (2001*). Total anthocyanins were expressed as cyaniding-3-glucoside for all of samples on dray weight basis, mg/100g. Absorbance was measured at 537 nm using spectronic 2000, spectrophotometer, Busch and lamb (USA).

Identification of individual phenolic compounds by HPLC

Phenolic compounds were identified by HPLC according to the method of *Goupy et al. (1999).* 5g of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Hewllet Packared (series 1050) equipped with autosamplling injector, solvent degasser, ultraviolet (UV) detector set at

280 nm and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of Hewllet Packared software, Germany.

Determination of antioxidant activity of the extracts:

Preparation of grape by-products extracts

Samples were air-dried and homogenized. Dry sample (5g) was placed in flask with 50 ml of extraction solution (80-20 methanol/ H_2O) according to *Vinson et al., (2001*). The mixture was placed in the dark at 4°C for 24 hrs. The supernatant was collected and replaced with an equal quantity of extraction solution, then placed in the dark at 4°C for a further 48 hrs. The two supernatants were mixed and extraction solution was added until a total volume of 100 ml was obtained. The solvent was removed and the extract was stored at -20 °C for further analysis.

***Determination of antioxidant activity using (DPPH) radical scavenging method*:**

Antioxidant activity of grape by-products samples (WGS, RGS, BGS and WG Seeds) was determined using the stable radical (DPPH) according to (*Brand – Williams et al., 1995*). The absorbance was read at 515 nm by Perkin Elmer spectrophotometer.

$$\% \text{ inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

$$\text{Antiradical efficiencies} = \frac{1}{EC50}$$

EC50 = extraction concentration providing 50% inhibition of the DPPH.

Determination of antioxidant activity in linoleic acid system

Antioxidant activity of grape by-products samples extracts (WGS, RGS, BGS and WG Seeds) was carried out by using the linoleic acid system (*Osawa and Namiki, 1981*) 200, 400, 800 ppm samples and BHA (200 ppm) were added to a solution mixture of linoleic acid (0.13ml), 99% ethanol (10ml) and 0.2M phosphate buffer (pH 7.0. 10ml). The total volume was adjusted to 25ml with distilled water. The solution was incubated at 40 ºC and the degree of oxidation was measured according to the thiocyanate method.

Oxidative stability of sunflower oil by different concentrations of grape by-products extracts:

Oxidative stability of sunflower oil at 100 °C by different concentrations of grape by-products extracts was measured using 679 Rancimat (Metrohm Ltd., CH.9100 Herisau, and Switzerland) Agric Res., Center, Giza at 100±2 °C. The sunflower oil free of additives was used as the substrate for oxidation studies (control Sample). Freeze dried extracts of WGS, RGS, BGS, WGSeeds at concentrations of (200, 400 and 800 ppm) and BHA were added the oil with the concentration 200 ppm. Ion products the volatile decomposition Products (mainly organic acid) are trapped a measuring detected with distilled water (60 ml) and continuously detected with a conductivity cell (conductivity range 25-200 us/cm) according to the method described by (*Gutteridge and Halliwell, 2000*).

2- Biological Evaluation

Experimental design

Seventy Male albino rats weighing 90- 120 grams were used for the study. They were purchased from Institute of Ophthalmology, Giza, Egypt. The animal housed individually in stainless steel under control condition at constant temperature (22 °C) and lighting (12 light- dark cycles). Rats were divided into 14 groups, five rats in each group and were fed the following diet for four weeks.

Group1: rats were fed the basal diet (control group) standard diet was prepared according to *Reeves et al., (1993)*.

Group2: rats were fed the basal diet containing 200ppm BHT

Groups 3, 4 and 5: rats were fed the basal diet containing 2%, 4% and 8% WGS powder

Groups 6, 7 and 8: rats were fed the basal diet containing 2%, 4% and 8% RGS powder

Groups 9, 10 and 11: rats were fed the basal diet containing 2%, 4% and 8% BGS powder

Groups 12, 13 and 14: rats were fed the basal diet containing 2%, 4% and 8% WG Seed

Each rat was weighted at the beginning and end of experimental. At the end of the experimental period (four weeks), rats were sacrificed after overnight fasting. Blood of each rat was collected and centrifuged at 300 rpm for 20 minutes to obtain the serum, which was kept at -20 °C until analysis.

Determination of lipid profile

Serum glucose, serum total cholesterol, serum triglycerides, HDL cholesterol and LDL cholesterol were determined as described by *Trinder, (1969*) *Richomand, (1973*); *Burstein et al., (1970); Wieland and Seidal, (1983) and Jacobs and Vandermark, (1960)*.

Determination of liver enzymes

ALT and AST were determined by the method of *Reitman and Franakal, 1957.*

Determination of antioxidant enzymes

Catalase and Glutathione reductase were determined by the method of *Aebi, 1984*; Goldberg and *Spooner, 1983*.

Statistical analysis

Data were evaluated statistically using analysis of variance. Duncan's multiple range tests at 5% level of significance was used to compare between means. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System (*SAS, 1996*).

3. Results and Discussions

Chemical composition of grape by-product (WGS, RGS, BGS and WG Seed) was determined. The obtained results are shown in Table (1).

It was noticed that the highest percentage of moisture (18.97%) and protein (10.146%) were observed of WGS. Meanwhile the highest percentage of Ash (8.36%) and total sugar (54.103%) obtained from BGS. Moreover WGSeed contained the highest percentage of Fat (10.38%) and total fiber (37.25%). These results are in line with those of *Schieber et al., (2002) and Zein et al., (2005*).

Gas liquid chromatography technique (GLC) was employed to study the fatty acid composition of WGSeeds. The results are shown in Table (2). These result agreements with *Beveridge et al., (2005*) reported that linoleic acid of seven different varieties of grape seed oil were ranged (66.8-73.6%). Oleic and palmitic acid were present as a major component (20.44% and 13.93%) after linoleic acid. These results are in line with that obtained by *Crews et al.,* (*2006). Lutterodt et al., (2011*) reported that the Egyptian grape seeds contained higher amount of oleic acid. Arachidic acid, Eurucic acid and palmitoleic acid were present as a minor components percentage of (0.17, 0.21 and 0.25%) respectively.

Total phenolic and total anthocyanins contents in WGS, RGS, BGS and WG Seeds were determined and the results are shown in Table (3) . The results indicated that the highest concentration of total phenolic compounds was obtained for WG Seeds (2536.5 mg/100g) followed by BGS and RGS (2070.02 and 511.23 mg/100g). While WGS had the lowest total phenolic compounds (296.27 mg/100g).

Negro et al., (2003) mentioned that the quantity of total phenolic substances and flavonoids contained in grape seed extract was higher than that obtained from marc and peel. In addition, *Anstasiadi, et al., (2010)* reported that grape seeds had a total phenolic contents ranged between 825.8 and 3313.5 mg/100g GAE while, the total phenolic contents for the same grape skins ranged between 64.5 and 351.97 mg/100 GAE.

Total anthocyanins of WGS, RGS, BGS and WG seed are shown in Table (1). The highest levels of total anthocyanin was obtained of BGS (300.37 mg/100g) followed by RGS (47.3 mg/100g), WG seed (13.64 mg/100g). Meanwhile WGS had the lowest total anthocyanins (4.09 mg/100g). Concentration of anthocyanin and phenolic compound was different among grape by-products. These results are resemblances with that established by *Pastrana- Bonilla et al., (2003),* for five bronze and five purple cultivars of muscadine grape skins and seeds in Georgia who mentioned that the concentration and total contents of anthocyanins and phenols varied among different varieties.

There are wide variations between the total phenolics contents of the different fruits or vegetables or even for the same fruits or vegetables reported by different authors. These differences may be due to the complexity of these groups of compounds, and the methods of extraction and analysis (*Bravo, 1998; Kalt et al., 2001 and Maier et al., 2009).*

Besides, phenolics contents of plant depend on a number of intrinsic (genus, species, cultivars) and extrinsic (agronomic, environmental, handling and storage) factors (*Toma´s-Barbera´n and Espin, 2001*). *Bozan et al., (2008*) studied the polyphenolic contents in the seeds of 11 red grape varieties cultivated in Turkey and found that the total phenolic content ranged from 79.2 to 154.6 mg GAE/ g seeds. While *Adamez et al., (2012)* found that the total phenolic content ranged between 6.04±0.6 GA g/L^{-1} for the seeds obtained from juice and 2.41±34 gL^{-1} GA for the seeds obtained from wine

Polyphenolic composition of extracts by HPLC

HPLC coupled with a UV-Vis detector was employed to separate and quantify phenolic compound from white, red, black grape skin and white grape seeds. The amounts of the different identified phenolic components are presented in Table (4). The major phenolic components in white grape skin were Di-OH cinammic acid, salicylic acid, Di-OH benzoic acid and synergic acid (4.91, 2.93, 2.90 and 2.45 ppm).

In red grape skin, the abundant compounds were pyrogalol, Di-OH cinammic acid, vanillic acid, synergic acid and catechol (11.41, 8.20, 3.6, 2.90 and 2.30 ppm, respectively) while, salicylic acid, Di-OH benzoic acid are not detected in the red grape skin. Concerning to the black grape skin, Trans 4OH-3CH-3O-Cinnammic acid, Di-OH Cinnammic acid, salicylic acid, P. OH benzoic acid and vanillic acid were the most abundant phenolic components (6.8, 6.7, 3.9, 3.6 and 2.7 ppm, respectively). In addition Table (4) shows that other phenolic compound such as Gallic acid, *P*-coumaric acid, procyanidin B1, B2 and B3 and catechin are also found with minor constituents in the three grape skins studied. The comparison among the polyphenolic profile of the three grape skin varieties studied revealed that the poly phenolic content varied with cultivar.

However, the red and black grape skin exhibit higher polyphenolic content as compared to the white grape skin. These finding are consistent with the previous work (*Berrin et al., 2008*) which noted that total monomeric and oligomeric flavanol

contents varied with variety and with the results obtained by *Anastasiadi, et al. (2010)* on polyphenolic composition involving skin of Greek grape cultivars. The difference in phenolic content and composition in the skin of grapes could be partly attributed to the genotype and environmental conditions (*Montealegre et al., 2006*), whereas wide ranges of grape skins contained lower amounts of procyanidin monomer with no significant differences among the genotypes (*Poudel et al., 2008*).

Data in Table (4) also revealed that they exhibit a very different qualitative and quantitative polyphenolic profile. Seeds are particularly rich in monomeric favan-2- ols (+) catechin and the dimeric procyanidin B1, B2 and B3. They also display a high level of Di-OH cinammic acid, salicylic acid. The quantity of the abundant phenolic components in the seeds were 521.80, 357.01, 269.70, 231.87, 185.40 and 174.10 ppm for (+) catechin, procyanidin B1, Di-OH cinammic acid, procyanidin B3, salicylic acid and procyanidin B2 respectively. These results are in accordance with the previous studies on polyphenolic composition involving seeds of Greek cultivars (*Guendez , et al., (2005a and 2005b*) which, noted that the most abundant polyphenolic compound in grape seeds extracts was catechin (189mg/100g) accounting for 49.8% of the TPC, followed by epicatechin (98.6mg/100g) and epecatechin gal late (35.5mg/100g seeds) the present results also in agreement with that obtained by *Anastasiadi, et al.,(2010)* and *Adamez et al., (2012*). In addition, flavonoids have been found to be the abundant phenolic compounds in grape seeds mainly catechin, epicatechin and epecatechin gallate and dimeric procyanidin B1 and B2 (*Naczk, et al., 2005*, *Maier et al., 2009 and Yi et al., 2009*).

On the other hand, our results are different from that reported by *Tounsi et al., (2009*) who reported that the most abundant polyphenolic compound in three different grape seeds varieties was quercetin accounting for 27.2, 48.8, and 28.4% of the total phenolic content of Muscat, Syrah and Carignan grape varieties, respectively. They also found that dimeric proanthocyandins B1 and B2 were minor constituents in all grape varieties studied.

Large difference in the phenolic compositions in different parts of the grape fruit have been also reported by *Pastrana- Bonilla et al. (2003*). Finally, the composition of phenolic in grape varies with variety, species and season conditions as well as environmental and management factors such as soil conditions, climate and crop load (*Tounsi et al., 2009*).

Scavenging effect of extracts on DPPH radical

The free radical scavenging activity of different grape by-product extracts was evaluated with the change of absorbance produced by reduction of DPPH. The results are summarized in Table (5). The high antioxidant capacity of all extracts has been observed and related to the presence of a mixture of polyphenolic compounds with good antioxidant activity. Seeds extract showed higher scavenging activity than all other extracts with EC50 0.259µg extract/µg DPPH followed by black grape skin extract (EC50 3.98µg extract/µg DPPH) and red grape skin extract (EC50 20.87µg extract/µg DPPH) while the white grape skin extract showed the lowest scavenging activity with EC50 28.91µg extract/µg DPPH. The potent and scavenging activity of the seeds extract is mainly attributed to its high contents of procyanidin B1 and B3 which have been assumed to be the most important radical scavengers in grape seeds extracts (*Guendez et al., 2005(b) and Maier et al., 2009*). However, the seeds extract was also characterized by high catechin content.

Our results indicated that the scavenging capacity of white and red grape skin extracts were dependent upon concentrations of the phenolic compound. On the other hand scavenging capacity of black grape skin grape seeds extracts increased with increasing concentration of black grape skin extract in the range 0 – 21.08 µg extract/µg DPPH grape seed extract up to 1.92 µg extract/µg DPPH after which scavenging effect on the DPPH radical was found to decrease. Thus, both black grape skin and grape seeds have very good antioxidant potential at lower concentrations and start showing prooxidant behavior at higher concentrations. Our results are consistent with that obtained by some investigators and disagree with others.

Some authors showed that a fine linear correlation exists between antioxidant capacity and total phenol contents in wine and wine by products (*Alonso et al., 2002; Ghiselli, et al.*, 1998 and Louli, *et al.,* 2004*).* The studies by *Jayaprakasha et al., (2003) and Adamez et al., 2012*) indicated that radical scavenging activity of the grape seeds extracts was dependent upon the contents of phenolic compound. While, a number of studies indicated that many of the dietary phenolic compounds have concentration-dependent antioxidant or prooxidant activities (*Yoshino and Murakami, 1998; Yen et al., 2002 and Maurya and Devasagayam*, *2010).* The beneficial effects of dietary antioxidants mainly focus on their defensive function against excessive oxidative damage induced (*Middleton et al., 2000*). .

Antioxidant activity of extracts from different grape skins and white grape seeds at different concentrations (200, 400 and 800ppm) were investigated in lenoleic acid system and the results are summarized in Table (6). It could be noticed that the antioxidant activity of grape skin and seeds extracts was high when used at low concentration 200ppm while the antioxidants were decreased with increasing the extract concentration up to 800ppm.

These results are in agreement with our previous result for DPPH.

Effect of extracts on oxidative stability of sunflower oil

Different concentrations of polyphenolic compounds extracted from white, red and black grape skin and white grape seeds were added to sunflower oil at concentrations of 200 and 400 ppm. In addition 200 ppm BHT was used as synthetic antioxidant. Oxidative stability of all samples was measured by rancimat method at 100 °C.

Table (7) and Figure (1) showed the results as induction period. The results indicated that BHT was superior to all natural extracts in agreement with (*Peschel et al., 2007*). When adding polyphenolic extractions, the results showed that BGS, (200ppm) had the highest stability with 12.9h followed by the WGS and RGS (12.8h, 12.8h) respectively with the same concentration and the same timeline while the WG seeds showed the lowest stability.

Effect of different grape by-product on body weight and lipid profile

Effects of different grape by-product on body weight are shown in Table (8). Data in Table (8), illustrated that there are no significant different $P<0.05$ in initial body weight and final body weight compared to the control group. There are no significant change was observed in body weight gain of all treatment compared to the control group except for red group skin at 4% and 8% BGS.

Data in Table (9) demonstrated that feeding rats on diet containing 200ppm BHT (synthetic antioxidant) showed significantly increase $P<0.05$ of total cholesterol (TC), LDL-c and Triglycerides (TG) compared to the control group. The rate of increase was 61.11%, 88.32% and 15.50% respectively compared to the control group.

Meanwhile, the addition of 2% WGS, RGS, BGS and WG Seeds of rats diet showed significant decrease P<0.05 of TC, LDL-c and TG compared to the control group. On the other hand, 4% (RGS, WGSeeds), 8% BGS and 2% WGSeeds showed the same effects as BHT. These results in accordance with the results obtained by (*Perez- Jimenez et al., 2008* and *Jiao et al., 2010*). reported that the supplementation with 0.5% or 1% grape seed proanthocyanidin and 7.5g/d grape antioxidant dietary fiber decrease total cholesterol and triglycerides. *Tebib et al., (1997)* found that 2% addition of seed extract to diet containing 1% cholesterol reduced plasma total cholesterol and LDL-C.

Feeding rats on diet containing 200ppm BHT and 4% (WGS, RGS, and BGS) showed that no significant change of HDL-c compared to the control group. The best results in serum HDL-c recorded for the group fed on diet treated daily with WG Seed in all levels followed by the group fed on diet treated daily with grape skin 8%, the group fed on diet treated daily with grape skin 4%. Our results are in line with (*Martin-Carron et al., 1999*) who indicated that HDL-cholesterol concentration was significantly higher in rats fed on dietary fiber and polyphenol -rich grape product than in the unsupplemented group.

Data in the same Table revealed that, serum Glucose increased gradually by increasing the levels of grape skin and seed, in the diet. On the other hand, the mean values of serum Glucose increased significantly in groups which treated with grape skin and seed, comparing with non treated groups. Increase of serum glucose may be due to grape pomace containing high level of sugars. These results are in agreement with Sayago – Ayerdi *et al.,* (2009) who mentioned that the grape pomace containing high level of sugar soluble (20.7±0.30g/kg).

Effect of grape by products on antioxidant and liver enzymes

Effect grape by-product on antioxidant enzymes are shown in Table (10). The results in Table (10) illustrated that treating rats with 200ppm BHT, WGS, RGS, BGS at 2% and 4% showed that no significant change ($P \leq 0.05$) in catalase enzyme activity, as compared to the control group. While, feeding rats on diet containing 8% (WGS, RGS, BGS) and WG Seed at different levels caused a significant increase in catalase enzyme activity. These results are disagreement with (*Alía et al., 2003*) who reported that antioxidant dietary fiber from grapes had no effect on the activity of catalase enzyme and there is an agreement with (*Xu et al., 2009*) who reported that grape seed extract increased the activity of catalase (CAT).

The data in this Table revealed that, glutathione enzyme activity in BGS 2% and WG Seed 2% groups increased significantly $P \leq 0.05$ (0.06±0.007a and 0.06±0.008a) respectively as compared to the groups fed on the same diets with other different levels of grape skin and seed. These results are in line (*Yousef and Romeo, 2004*) showed that polymeric grape seed tannin in the diet increase total glutathione level in blood. These results not agreement with (*Yousef et al., 2009*) who reported that grape seed proanthocyanidin extract decrease Glutathione enzyme activity.

Effect of feeding rats on diets containing different levels from grape skin and seed (2%, 4% and 8%) on the activities of some liver enzymes AST and ALT in serum of rats was illustrated in Table (10)

The results indicated that feeding rats on diet containing 4,8% grape skin and seed, decreased liver function (ALT) by about 63.63% than that of the control group. Meanwhile, 2% grape skin and seeds showed a significant increase of liver enzymes. These results are agreement with (*Yousef et al., 2009*) who reported that grape seed

proanthocyanidin extract caused significant increase in AST and ALT. These results approved by *Kotamballi et al., (2002)* indicate that the grape pomace MeOH extract is capable of protecting the activities of hepatic enzymes, which play important roles in combating the reactive oxygen species.

Table (1): Major chemical constituents (g/100g dry matter) of grapes skin and grape white seeds

Constituents (%)	Grape skin			White grape Seeds
	White	Red	Black	
Moisture	18.97	7.28	18.21	8.38
Fat	3.93	6.43	1.89	10.38
Protein	10.146	6.999	7.872	9.076
Total Fibers	10.56	7.335	9.565	37.25
Ash	6.35	2.94	8.36	2.38
Total Sugar	50.044	69.016	54.103	32.534

Table (2): Fatty acid profile of grape seeds oil by GLC

Fatty acids		Percentage %
Saturated fatty acids:		13.93
Palmitic acid	C16:0	8.59
Stearic acid	C18:0	5.16
Arachidic acid	C20:0	0.17
Unsaturated fatty acids:		86.07
Palmitoleic acid	C16:1	0.25
Olic acid	C18:1	20.44
Linoleic acid	C18:2	64.72
Linolenic acid	C18:3	0.43
Eurucic acid	C20:1	0.23

Table (3): Total phenolic compounds, total anthocynins, efficient concentration and antiradical efficiencies of different grape skin and white grape seeds

Samples	Total phenolic compounds mg/100g	Total anthocyanin mg/100g	Efficient concentration (EC50)	Antiradical efficiencies (AE)
WGS	296.27	4.09	28.91	0.043
RGS	511.23	47.3	20.85	0.048
BGS	2070.02	300.37	3.98	0.25
WG Seeds	2536.5	13.64	0.26	4.00

Table (4): Polyphenolic composition of extracts from white, red, black grape skins and white grape seeds by HPLC

Phenolic compounds(ppm)		Test results			
		White grape skin	Red grape skin	Black grape skin	White grape Seeds
1	Gallic	1.04	0.20	0.70	11.07
2	Catechol	0.96	2.30	2.40	20.20
3	Pyrogalol	2.07	11.41	---	---
4	Di-OH Benzoic	2.90	---	1.1	35.07
5	P.OH Benzoic	0.107	0.47	3.60	1.10
6	Catechin	0.84	1.7	1.9	521.80
7	Vanillic	1.25	3.60	2.70	63.40
8	Procyanidin B1	1.61	1.07	1.72	357.01
9	P-Coumaric	0.26	0.69	0.90	23.30
10	Chrisin	0.83	0.12	0.40	---
11	Chlorogenic	---	2.17	1.004	66.30
12	Synergic	2.45	2.90	1.90	64.20
13	Trans-4OH-3CH-3O-Cinnammic	0.75	1.40	6.80	---
14	Salicylic	2.93	---	3.90	185.40
15	Di-OH Cinnammic	4.91	8.20	6.70	269.70
16	Hespertin	0.21	0.50	1.20	28.80
17	Procyanidin B3	1.05	1.55	1.19	231.87
18	Procyanidin B2	1.14	1.44	1.69	174.10

Table (5): The effects of phenolic compound of different grapes skin and white grape Seeds on remaining percentage of DPPH and inhibition ratio of DPPH µg/µg.

White Grape skin		Red Grape skin		Black Grape skin		White Grape Seed	
Conc. µg/µg	Inhibition Ratio%	Conc. µg/µg	Inhibition Ratio%	Conc. µg/µg	Inhibition Ratio%	Conc. µg/µg	Inhibition Ratio%
12.86	30.0	9.06	22.96	1.07	31.07	0.26	51.18
16.08	31.4	11.33	28.90	1.68	33.02	0.34	73.46
18.37	37.4	12.92	34.28	2.08	41.87	0.51	75.83
21.04	38.2	15.10	42.18	2.7	46.44	1.00	86.89
21.44	40.9	16.31	43.67	3.98	50.24	1.92	97.20
25.73	44.5	20.87	50.07	7.32	74.40	3.52	86.73
42.88	63.2	26.23	55.76	21.08	84.20	4.23	84.73
64.33	72.8	45.33	66.03	43.92	68.99	21.2	68.29

Table (6): Antioxidant activity of different grape skin and white grape seed in linoleic acid system

Samples	Absorbance at 515nm Storage time (days)							
	Zero	1	3	5	8	11	14	16
Control	0.244	0.269	0.288	0.294	0.305	0.337	0.348	0.358
BHT(200ppm)	0.244	0.257	0.264	0.271	0.276	0.281	0.288	0.298
WGS 2%	0.244	0.258	0.262	0.296	0.278	0.268	0.293	0.306
WGS 4%	0.244	0.258	0.278	0.285	0.297	0.305	0.316	0.323
WGS 8%	0.244	0.272	0.294	0.312	0.322	0.339	0.342	0.359
RGS 2%	0.244	0.245	0.266	0.273	0.279	0.284	0.291	0.305
RGS 4%	0.244	0.267	0.290	0.292	0.306	0.338	0.349	0.361
RGS 8%	0.244	0.271	0.299	0.328	0.345	0.352	0.359	0.374
BGS 2%	0.244	0.252	0.260	0.271	0.278	0.282	0.29	0.302
BGS 4%	0.244	0.259	0.272	0.279	0.295	0.354	0.352	0.365
BGS 8%	0.244	0.268	0.297	0.302	0.348	0.355	0.361	0.379
WG Seed 2%	0.244	0.256	0.267	0.275	0.28	0.289	0.298	0.314
WG Seed 4%	0.244	0.262	0.309	0.313	0.338	0.398	0.427	0.447
WG Seed 8%	0.244	0.278	0.332	0.352	0.378	0.502	0.531	0.563

Table (7): The effect of addition different grape by-products samples on Oxidative stability of sunflower oil at 100 °C by Rancimat

Samples	Oxidative stability At 100°C
Control	11.9
BHT	14.6
WGS 200ppm	12.8
RGS 200ppm	12.8
BGS 200 ppm	12.9
WG Seed200ppm	12.2
WGS 400ppm	12.4
RGS 400ppm	11.8
BGS 400 ppm	11.6
WG Seeds 400ppm	11.4

Table (8): Effect of different types grape skin and white grape seed of grape on body weights

groups	Initial body Weight (gm) Mean ±SD	final body weight (gm) Mean ±SD	Body weight gain (gm) Mean ±SD
1-Control	107.00 ± 10.95^{a}	157.60 ± 13.16^{ab}	50.60 ± 7.12^{bc}
2-BHT	107.00 ± 10.95^{a}	149.80 ± 8.13^{ab}	42.80 ± 5.87^{c}
3-WGS 2%	107.00 ± 10.95^{a}	150.00 ± 7.07^{ab}	43.00 ± 7.34^{c}
4-WGS 4%	107.00 ± 10.95^{a}	165.60 ± 7.82^{ab}	58.60 ± 6.07^{b}
5-WGS 8%	107.00 ± 10.95^{a}	164.60 ± 14.06^{ab}	57.60 ± 7.52^{b}
6-RGS 2%	107.00 ± 10.95^{a}	156.20 ± 19.1^{ab}	49.20 ± 11.77^{bc}
7- RGS 4%	107.00 ± 10.95^{a}	145.20 ± 7.91^{b}	38.20 ± 6.80^{d}
8-RGS 8%	107.00 ± 10.95^{a}	157.40 ± 10.62^{ab}	50.40 ± 8.65^{bc}
9-BGS 2%	104.00 ± 10.83^{ab}	157.00 ± 15.47^{ab}	53.00 ± 5.37^{bc}
10-BGS 4%	104.00 ± 10.83^{ab}	161.00 ± 10.04^{ab}	57.00 ± 8.59^{b}
11-BGS 8%	104.00 ± 10.83^{ab}	166.40 ± 9.28^{a}	62.40 ± 4.06^{a}
12-WG Seed 2%	104.00 ± 8.94^{ab}	163.00 ± 14.81^{ab}	59.00 ± 5.64^{b}
13- WG Seed 4%	104.00 ± 10.83^{ab}	160.40 ± 14.08^{ab}	56.40 ± 6.50^{b}
14- WG Seed 8%	108.00 ± 16.43^{a}	153.60 ± 14.39^{ab}	45.60 ± 6.26^{c}

Table (9): Effect of different types grape skin and white grape seed on lipid profile and Glucose

groups	Total cholesterol mg/dl means ± SDM	HDL- C mg/dl means ± SDM	LDL-c mg/dl means ± SDM	VLDL-C mg/dl means ± SDM	Triglycerides mg/dl means ± SDM	Glucose mg/dl means ± SDM
1- Control	79.90±17.80 ^{c}	44.41±0.89 ^{d}	58.60±19.90 ^{f}	23.12±1.68 ^{ab}	115.62±8.43 ^{b}	20.26±8.92 ^{e}
2- BHT	128.73±13.53 ^{a}	45.08±2.17 ^{d}	110.36±10.24 ^{a}	26.70±5.26 ^{a}	133.54±26.33 ^{a}	27.53±14.41 ^{d}
3- WGS 2%	58.33±24.00 ^{d}	37.03±6.67 ^{e}	71.60±1.06 ^{d}	22.86±3.04 ^{b}	114.31±15.20 ^{b}	27.53±7.50 ^{d}
4- WGS 4%	93.40±10.17 ^{bc}	41.69±5.79 ^{de}	75.23±12.22 ^{d}	23.51±4.16 ^{b}	117.59±20.84 ^{b}	40.00±6.24 ^{b}
5- WGS 8%	109.00±18.14 ^{b}	48.63±8.41 ^{bc}	87.60±15.89 ^{c}	26.23±1.05 ^{a}	136.17±5.29 ^{a}	42.00±7.00 ^{b}
6- RGS 2%	109.43±20.04 ^{b}	42.17±7.66 ^{c}	85.30±17.35 ^{cd}	23.03±6.03 ^{ab}	115.18±17.41 ^{b}	39.33±3.21 ^{b}
7- RGS 4%	126.06±20.05 ^{a}	44.71±0.57 ^{d}	71.38±20.33 ^{ab}	22.03±1.59 ^{b}	110.16±7.95 ^{c}	41.16±9.64 ^{b}
8- RGS 8%	94.33±10.26 ^{bc}	48.10±5.91 ^{bc}	103.58±13.72 ^{d}	25.35±2.41 ^{a}	126.77±12.0 ^{ab}	42.33±4.37 ^{b}
9- BGS 2%	94.66±22.53 ^{bc}	24.52±0.98 ^{f}	53.09±23.72 ^{b}	22.95±0.59 ^{b}	114.75±2.95 ^{b}	42.66±16.79 ^{b}
10- BGS 4%	93.13±6.37 ^{bc}	41.45±4.63 ^{de}	93.25±6.27 ^{j}	25.57±0.60 ^{a}	127.86±3.00 ^{ab}	52.46±12.62 ^{ab}
11- BGS 8%	121.36±1.35 ^{a}	62.39±2.84 ^{de}	105.45±3.13 ^{ab}	25.48±1.82 ^{a}	127.43±9.12 ^{ab}	59.28±8.53 ^{a}
12- W.G Seed 2%	128.50±8.94 ^{a}	63.28±6.20 ^{abc}	91.22±5.75 ^{bc}	26.01±1.64 ^{a}	129.18±9.04 ^{ab}	25.33±5.82 ^{d}
13- WG Seed 4%	118.13±9.52 ^{ab}	77.53±3.62 ^{a}	63.81±11.78 ^{e}	23.21±0.46 ^{ab}	116.06±2.30 ^{b}	33.33±3.49 ^{c}
14- WG Seed 8%	93.43±5.30 ^{bc}	70.13±1.13 ^{a}	46.12±4.19 ^{h}	22.81±1.16 ^{b}	114.09±5.82 ^{b}	34.33±3.39 ^{c}

Table (10): Effect of different types grape skin and white grape seed on Antioxidant enzymes activity and liver function

groups	Enzyme Activity		Liver function	
	Catalase mM/L Mean ± SD	Glutathione mM/L Mean ± SD	AST µg/dl Mean ± SD	ALT µg/dl Mean ± SD
1- Control	87.4±2.16 ^{b}	0.04±0.001 ^{c}	38.76±1.26 ^{c}	69.28±2.0 ^{c}
2- BHT	85.23±5.81 ^{b}	0.03±0.004 ^{d}	41.31±3.54 ^{bc}	77.71±3.85 ^{b}
3-WGS 2%	85.32±4.77 ^{b}	0.04±0.003 ^{c}	41.03±2.73 ^{bc}	81.06±5.10 ^{ab}
4- WGS 4%	86.46±3.65 ^{b}	0.04±0.001 ^{d}	34.40±1.96 ^{d}	68.56±7.90 ^{b}
5- WGS 8%	101.90±5.53 ^{a}	0.04±0.001 ^{c}	32.67±3.27 ^{d}	59.77±3.15 ^{d}
6- RGS 2%	85.24±2.64 ^{b}	0.05±0.001 ^{bc}	43.16±2.78 ^{ab}	82.66±3.21 ^{ab}
7-RGS 4%	89.56±0.83 ^{b}	0.04±0.002 ^{c}	34.04±2.26 ^{d}	46.38±3.41 ^{e}
8- RGS 8%	99.97±2.14 ^{a}	0.04±0.0005 ^{d}	28.40±0.94 ^{e}	35.83±2.17 ^{f}
9- BGS 2%	89.04±0.42 ^{b}	0.06±0.007 ^{a}	45.67±2.49 ^{a}	86.87±3.54 ^{a}
10-BGS 4%	89.68±0.34 ^{b}	0.05±0.005 ^{b}	33.45±1.86 ^{d}	43.77±3.37 ^{e}
11- BGS 8%	102.74±3.07 ^{a}	0.04±0.003 ^{c}	26.26±2.73 ^{e}	35.09±1.71 ^{f}
12-WG Seed 2%	99.11±0.76 ^{a}	0.06±0.008 ^{a}	41.72±2.58 ^{abc}	83.11±4.06 ^{ab}
13- WG Seed 4%	100.02±0.49 ^{a}	0.05±0.005 ^{b}	32.99±1.62 ^{d}	36.95±1.75 ^{f}
14- WG Seed 8%	103.46±1.04 ^{a}	0.04±0.003 ^{c}	26.20±0.79 ^{e}	32.98±2.92 ^{f}

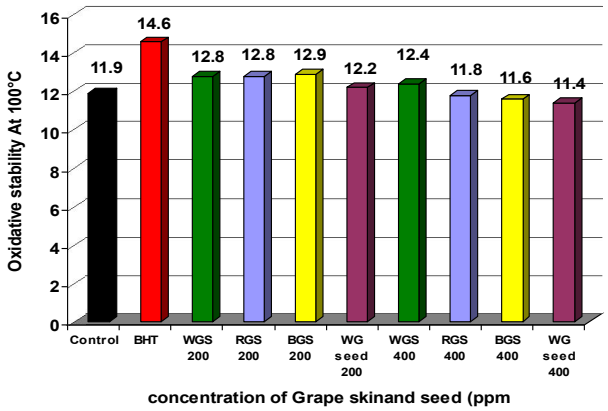

Fig (1): The effect of addition of different grape by-products samples on Oxidative stability of sunflower oil at 100°C by Rancimat

Conclusion

It could be concluded that the main phenolic compound in the three grape skins was Di-OH-cinamic acid. In the contrast, seeds are particularly rich in monomeric favan-2- ols (+) catechin and the dimeric procyanidin B1, B2 and B3. They also display a high level of Di-OH cinammic acid, salicylic acid. Our results indicated that all extracts showed remarkable DPPH radical scavenging activities with EC50 values ranged from 0.26-26.91µg extract/µg DPPH. The grape by-products (skins and seeds) could be used as good sources for natural antioxidant especially at low concentration.

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References

1. Adamez, J. D.; Samino. E. G.; Sanchez, E. V. and Gomez, D. (2012): *In vivo* estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape seeds (*Vitis vinifera L.*). Food Control, 24: 136 - 141.
2. Aebi, H. (1984): Method for the measurement of catalase in human fluid methods. Enzymology, 105: 121- 126.
3. Alia, M.; Horcajo, C.; Bravo, L. and Goya, L. (2003): Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. Nutrition Research, 23 (9): 1251- 1267.
4. Alonso, A. M.; Guillten, D. A.; Barroso, C. G.; Puertas, B. and Garcıa, A. (2002): Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. Journal of Agricultural and Food Chemistry, 50:5832–5836.
5. Anon (2003): Overview of antioxidants, AGRO Food Industry, 51-53.
6. Anstasiadi, M.; Pratsinis, H.; Kletsas, D.; Skaltsounis, A. and Haroutounian, S. (2010): Bioactive non-colored polyphenols content of grapes, wines and vilification by-products: Evaluation of the antioxidant activities of their extracts. Food Research International, 43:805-813.
7. AOAC (2000): Association of Official Analytical Chemists. 14th ed., Maryland, D.C.,USA
8. Asami K.D.; Hong, Y.J.; Barrett, M.D. and Mitchell, E.A. (2003): Composition of total phenolic and ascorbic acid content of freeze dried and air-dried Marino-berry, strawberry and corn grown using conventional, organic and sustainable agricultural practices. J. Agric. Food Chem., 51:1237-1241.
9. Balasundran, N., Sundram, K. and Samman, S. (2006): Phenolic compounds in plants by products: antioxidant activity, occurrence and potential uses. Food Chemistry, 99: 191- 203.
10. Baydar, N.G.; Ozkan G. and Yasser S. (2007): Evaluation of the antiradical and antioxidant potential of grape extracts. Food Control, 18:1131-1136.
11. Berrin, B.; Kassel, G. and Derya, T. (2008): Study of polyphenol content in the seeds of red grape (*Vitis vinifera L.*) verities cultivated in Turkey and their antiradical activity. Food Chemistry, 56 (12): 312- 327.
12. Beveridge, T. H. J.; Girard, B.; Kopp. T. and Drover, J. C. G. (2005): Yield and composition of grape seed oils extracted by supercritical carbon dioxide and petroleum ether: Varietals effect. Journal of Agricultural and Food Chemistry, 53: 1799-1804.
13. Bozan, B.; Tosun. G. and Ozone, D. (2008): Study of polyphenols content in the seeds of red grape (*Vitis vinifera, L.*) Varieties cultivated in Turkey and their antiradical activity. Food Chemistry, 109: 426- 430.
14. Brand – Williams, W.; Duvalier, M. E. and C. Berset (1995): Use of a free radical method to evaluate antioxidant activity. Food Science and Technology, 28:25-30.
15. Bravo, L. (1998): Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. Nutrition Reviews, 56: 317-333.
16. Burstein, M.; Scholinck, H. R. and Haarfin, R. (1970): Raped method for isolation of lipoprotein from human serum by precipitation with polyanion. Journal of Lipid Research, 11: 538- 595.
17. Crews, C.; Houg, P.; Godward, J.; Brereton, P. ; Lees, M.; Guiet, S. (2006): Quantitation of the main constituents of some authentic grape- seed oils of different origin. Journal of Agricultural and Food Chemistry, 54: 6261- 6265.
18. Farag, R. S.; Hallabo, S. A. S.; Hewedi, F. M. and Basyon, A. E. (1986): Chemical Evaluation of Rape seed. Fette Seifen Anstrichmittel, 88 (10): 391- 397.
19. Felice, F. ; Zambito, Y., Colo, G. D.; D'Onofrio, C. ; Fausto, C. ; Balbarini , D. And Stefano, A. R. D. (2012): Red grape skin and seeds polyphenols: Evidence of their protective effects on endothelial progenitor cells and improvement of their intestinal absorption. European Journal of Pharmaceutics and Biopharamacetics, 80: 176 - 184.
20. Ghiselli, A.; Nardini, M.; Baldi, A. and Scaccini, C. (1998): Antioxidant activity of different phenolic fractions separated from Italian red wine. Journal of Agricultural and Food Chemistry, 46: 361–367.
21. Goldberg, D. M. and Spooner, R. J. (1983): In Methods of Enzymatic Analysis (Bergmeyen, H. V. ed) 3rd edn. Vol3, pp 258- 265, Verlog Chemie, Deerfield beach, FI.
22. Goupy, P.; Hugues, M.; Biovin, P. and Amiot, M. J. (1999): Antioxidant composition and activity of barley (*Horden vulgar*) and malt extract and of isolated phenolic compounds. J. Sc. Food Agric., 79: 1625- 1634.
23. Guendez, R.; Kallithraka, S.; Makris, D. P. and Kefalas, P. (2005a): An analytical survey of the polyphenols seeds of varieties of grape (Vitis vinifera sp.) cultivated in Greece. Implication for exploitation as a source of value- added phytochemicals. Phytochemical Analysis, 16 (1): 17- 23.
24. Guendez, R.; Kallithraka, S.; Makris, D. P. and Kefalas, P. (2005b): Determination of low molecular weight polyphenolic constituents in grape (*Vitis vinifera sp*) seed extracts: Correlation with antiradical activity. Food Chemistry, 89: 1-9.
25. Gutteridge, J. M. and Halliwell. B. (2000): Free radical and antioxidants in the year 2000. A historical look to future. Annals of the New York Academy of Sciences, 899: 136- 147.
26. Harboner, J. B. (1994): In polyphenols 94, proceeding of the 17th International conference on polyphenols (Brouillared R. and Scalbert, A., eds) pp. 19- 26. INR Editions, Paris, France
27. Heim, K. E.; Tagliaferro, A. R. and Bobilya, D. J. (2002): Flavonoid antioxidants: chemistry, metabolism and structure-activity relationship. The Journal of Nutrition Biochemistry, 13: 572- 584.
28. Jacobs, N. and Vandermark, P. J. (1960): Determination of serum triacylglycerol. Archives of Biochemistry Biophysics, 88: 250- 261
29. Jayaprakasha, G. K.; Selvi, T. and Sakariah, K. K. (2003): Antibacterial and antioxidant activities of grape seed extracts. Food Research International, 36: 117–122.
30. Jiao, R.; Zhang, Z.; Yuc, H.; Huang, Y. and Chen, Z. (2010): Hypocholesterolemic activity of grape seed Proanthocyanidin in mediated by enhancement of bile acid extraction and up-regulation of CYP7AI. Journal of Nutrition Biochemistry, 21: 1134- 1139.
31. Kalt, W.; Ryan, D. A. J.; Duy, J. C.; Prior, R. L.; Ehlenfeldt, M. K. and Kloet, S. P. V. (2001): Interspecific variation in anthocyanins, phenolics and antioxidant capacity among genotypes of high bush and low bush blueberries (Vaccinium Section Cyanococcus SPP.). Journal of Agricultural and Food Chemistry, 49: 4761- 4767.
32. Kotamballi, N.; Murthy, C.; Singh, R.P. and Jayaprakasha, G.K. (2002): Antioxidant activities of grape (*Vitis vinifera*) pomace extracts. J. Agric. Food Chem., 50 (21): 5909-5914.
33. Lafka, T. I., Sinnanoglou, V. and Lazos, E. S. (2007): On the extraction and antioxidant activity of phenolic compounds from wine industry. Food Chemistry, 104: 1206- 1214.
34. Larson, R. A. (1988): The antioxidants of higher plants. Photochemistry, (27): 4, 969-978.
35. Liuis, L., Munoz, M.; Noues, M. R. ; Sanchez- Martos, V. ; Romeu, M.; Giralt, M. ; Valls, J. and Sola, R. (2011): Toxicology evaluation of a procyanidin rich extract from grape skins and seeds. Food and Chemical Toxicology, 49: 1450-1454.
36. Louli, V.; Ragoussis, N. and Magoulas, K. (2004): Recovery of phenolic antioxidants from wine industry by-products. Bioresearch Technology, 92: 201–208.

37. Lutterodt, H.; Slavin. M.; Whent, M.; Turner, E. and Yu, L. (2011): Fatty acid composition, oxidative stability, antioxidant and anti proliferative properties of selected cold- pressed grape seed oils and flours. Food Chemistry, 128: 391 – 399.
38. Makris, D.P.; Boskou, G. and Andrikopoulos, N.K. (2007): Polyphenolic content in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. Journal of Food Composition and Analysis, 20: 125–132.
39. Maier, T., Schieber, A., Kemmerer, D. R. and Carle, R. (2009): Residues of grape (Vitis Vinifera L.) seed oil production as a valuable source of phenolic antioxidants. Food Chemistry, 112 (3): 551z- 559z.
40. Martin- Carron, N.; Corti, L. ; Larrauri, J. A. ; Garcia- Alonso, A. and Saura- Calixto, F. (1999): Reduction in serum total and LDL Cholesterol Concentration by a dietary fiber and polyphenol-rich grape product in hypercholesterolemic rats. Nutrition Research, 19 (9): 1371- 1381.
41. Maurya, D. K. and Devasagayam, T. P. A. (2010): Antioxidant and prooxidant nature of hydroxycinnamic acid derivatives ferulic and caffeic acids. Food and Chemical Toxicology, 48: 3369–3373.
42. Middleton, J. E.; Kandaswami, C. and Theoharides, T. C. (2000): The effects of plant flavonoids on mammalian cells: implication for inflammation, heart disease and cancer. Pharmacology Rev., 52: 673 – 751.
43. Millard, M.; Scum, M.H. and Meydanis, N. (1996): Antioxidant activity of barley and malt relationship with phenolic content. Food Science and Technology. 29: 238-244.
44. Mohamed, E. M. and Girgis. A. Y. (2005): utilization of mango seed kernels for improving stability of some oils and biscuit production. Journal of Agricultural Science, Mansoura University, 30 (8): 4643- 4654.
45. Monica D.; Anthon Ella R.; Giuilia C.; Amgela A.; Alessandra L.; Francesco V.; Paola M. and Assunta M. (2007): Protective effect of olive oil minor polar components against oxidative damage in rats treated with ferric nitrilotriacetate. Food Chemical Toxicology, 45: 233-240.
46. Montealegre, R. ; Romero, P. R.; Chacon- Vozmediano, J. L.; Gascuena, M,; Garcia, J. And Romero, E. (2006): Phenolic compound in skin and seeds of ten grape Vitis vinifera varities grown in a warm climate. J. Food Comp. Anal., 19: 687- 693.
47. Naczk, M.; Amarowicz, R.; Zadernowski, R. and Shahidi, F. (2005): In F. Shahidi and C.T. Ho {Eds}. Phenolics in food and natural health products. ACS Symposium Series 909 (PP. 57-66). Washington, D. C: American Chemical Society.
48. Negro, C.; Tommasi, L. and Micelles, A. (2003): Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour Techno, 87: 41–44.
49. Osawa, T. and Namiki, M. (1981): A novel type of antioxidant isolated from leaf wax of eucalyptus leaves. Agric. Biol. Chem., 45: 735-739.
50. Palma, M.; Pinero, Z. and Barroso, C. G. (2001): Stability of phenolic compounds during extraction with superheated solvents. Journal of Chromatography A, 921: 169–174.
51. Pastrana-Bonilla, E.; Akoh, C. C.; Sellappan, S. and Krewer, G. (2003): Phenolic content and antioxidant capacity of muscadine grapes. Journal of Agricultural and Food Chemistry, 51(18): 5497–5503.
52. Perez- Jimenez, J., Serrano, J.; Tabernero, M.; Arranz, M. S.; Diaz-Rubio.; Garcia-Diz, L.; Coni, I. and Saura- Calixto, F. (2008): Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. Journal of Nutrition, 24: 646 – 653.
53. Peschel, W.; Dieckmann, W.; Sonnenschein, M. and Plescher, A. (2007): High antioxidant potential of pressing residues from evening primrose in comparison to other oilseed cakes and plant antioxidants. Industrial Crops and Products, 25: 44- 54.
54. Poudel, P. R. Tamura, H.; Kataoka, I. and Mochioka, R. (2008): Phenolic compounds and antioxidant activities of skin and seeds of five wild grapes and two hybrids native to Japan. Journal of Food Composition and analysis, 21: 622- 625.
55. Pratt, D. E. and Hudson, B. J. F. (1990): Natural antioxidants, not exploited commercially. In: Food antioxidants, Hudson, B.J.F. Ed, Elsevier, London, UK pp. 171-191.
56. Puupponen-Pimiä, R.; Nohynek, L.; Hartmann-Schmidlin, S.; Kähk○nen, M.; Heinonen, M.; Mättä-Riihinen, K. and Oksman-Caldentey, K.M. (2005): Berry phenolics selectively inhibit the growth of intestinal pathogens. J. Appl. Microbiol., 98: 991–1000.
57. Reeves, R. G.; Nielsen, F. H. and Iahey, C. (1993): AIN-93 purified diets for laboratory rodents. J Nutr., 123: 1939.
58. Reitman, S. and Franakal, S. (1957): A calorimetric method for determination of serum AST. American Journal Clinical Pathology, 18: 16.
59. Richmond, W. (1973): Preparation and properties of a cholesterol oxidase from Nacardia Sp. Enzymatic assay of total cholesterol in serum. Clinical Chemistry, 19 (12): 1350- 1356.
60. SAS program (1996): SAS/STAT User Guide Release 6.12 edition. Cary, NC, USA: SAS Inst. Inc.
61. Sayago- Ayerdi, S. G., Brines, A. and I. I. (2009): Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburger. LWT- Food Science and Technology, 42: 971- 976.
62. Schieber, A.; Muller, D.; Rohrig, G. and Carle, R. (2002): Influence of grape cultivar and processing method on the quality of cold- pressed grape seed oils, Mitteilugen Klosterneuburg, 52 (1-2), 29-33.
63. Tang, S; Kerry, J. P.; Sheehan, D. Buckley, D. J. and Morrissey. P. A. (2001): Antioxidant effect of added tea catechins on susceptibility of cooked red meat, poultry and fish patties to lipid oxidation. Food Research International, 34: 651-657
64. Tebib, K.; Rouanet, J. M. and Besancon, P. (1997): Antioxidant effects of dietary polymeric grape seed tannins in tissues of rats fed a high cholesterol vitamin E deficiency diet. Food Chemistry, 59 (1): 135- 141.
65. Toma´s-Barbera´n, F. A. and Espin, J. C. (2001): Phenolic compounds and related enzymes as determinants of quality of fruits and vegetables. Journal of the Science of Food and Agriculture, 81:853–876.
66. Tounsi, M. S.; Ouerghemmi, I.; Wanes', W. A.; Ksourib, R.; Zemni, H.; Marzouka, B. and Kchouk, M. E. (2009): Valorization of three varieties of grape. Industrial Crops and Products, 30:292–296.
67. Trinder, P. (1969): Determination of glucose in blood. Annals Clinical Chemistry, 6: 24.
68. Valiente, C.; Arrigoni, E.; Esteban, R. M. and Amado, R. (1995): Grape pomace as a potential food fiber. Journal of Food Science, 60 (4): 818–820.
69. Vinson, J. A.; Su, X.; Zubik, L. and Bose, P. (2001): Phenol antioxidant quantity and quality in foods: fruit. Journal of Agricultural and food Chemistry, 49: 5315- 5321.
70. Wieland, H. and Sedial, D. (1983): A Simple specific method for precipitation of low density lipoprotein. Journal of Lipid Research, 24: 904- 909.
71. Worlstad, R.E. and Giusti M. M. (2001): Characterization and measurement of anthocyanins by UV- Vis Spectroscopy-chemistry. Wiley INC, New York.
72. Xu, W.; Peng, X. ; Luo. Y. ; Wang, J.; Guo, X. and Huang, K. (2009): Physiological and biochemical responses of grape fruit seed extract dip on Red globe grape. LWT. Food Science and Technology, 42: 471- 476.
73. Yen, G., C.; Duh, P. D. and Tsai, H., L. (2002): Antioxidant and prooxidant properties of ascorbic acid and Gallic acid. Food Chemistry, 79:307-313.
74. Yi, C.; Shi , J.; Kramer , J.; Xue , S.; Jiang , Y.; Zhang , M.; Mad, Y. and Pohorly, J.(2009): Fatty acid composition and phenolic antioxidants of winemaking pomace powder. Food Chemistry, 114: 570–576.
75. Yilmaz, Y. and Toledo, R. T. (2004): Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and Gallic Acid. Journal of Agricultural and Food Chemistry, 52: 255–260.
76. Yoshino, M. and Murakami, K. (1998): Interaction of iron with polyphenolic compounds: application to antioxidant characterization. Anal. Biochem., 257 (1): 40–44.
77. Yousef, M.I.; Saad, A.A. and El-Shennawy, L.K. (2009): Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food and Chemical Toxicology, 47: 1176–1183.
78. Yousuf, Y. and Romeo, T. T. (2004): Health aspects of functional grape seed constituents. Triends in Food Science & Technology, 45: 422- 435.
79. Zein, R.E.; El-Bagoury, A.A. and Kassab, H.E. (2005): Chemical and nutritional studies on mango seed kernels. Journal of American Oil Chemists Society, 64 (6): 870- 873.