http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (3): 403-408, 2007
ISSN 1028-8880

Screening of Antioxidative Properties and Total Phenolic Compounds of Various Extracts of Three Different Seed of Grape Varieties (*Vitis vinifera* L.) From Turkish Flora

[1]Mustafa Kelen and [2]Bektas Tepe
[1]Department of Horticulture, Faculty of Agriculture,
Suleyman Demirel University, 32260 Isparta, Turkey
[2]Department of Biology, Faculty of Science and Literature,
Cumhuriyet University, 58140 Sivas, Turkey

Abstract: This study was designed to examine the *in vitro* antioxidant activities and total phenolic contents of various extracts prepared by using solvents of varying polarity from three different seed of grape varieties (*Vitis vinifera* L.) of which local names are Siyah gemre, Adana Karasi and Tilki kuyrugu in Turkish folk medicine, respectively. The extracts were screened for their possible antioxidant activity by two complementary test systems namely DPPH free radical scavenging and β-carotene/linoleic acid. In the first case, polar fractions of the methanol extracts of grape seeds exerted excellent activity patterns than those of non-polar fractions, while hexane and dichloromethane extracts did not exhibited activity. Among the polar ones, the most active extract was Adana karasi (5.90±0.20 μg mL^{-1}), followed by Tilki kuyrugu and Siyah gemre (6.40±0.50 μg mL^{-1} and 6.90±0.40 μg mL^{-1}, respectively). In DPPH system, grape seeds exerted two-fold greater antioxidant activity than that of synthetic antioxidant BHT. In β-carotene/linoleic acid test system, inhibition of linoleic acid oxidation was effectively achieved by polar and non-polar extracts of Siyah gemre. In this system, polar extracts exhibited greater antioxidant activity than those of non-polar ones, whereas hexane and dichloromethane extracts had no activity. The amount of total phenolics was highest in polar and non-polar extracts of grape seeds. Especially, a positive correlation was observed between total phenolic content and antioxidant activity of polar extracts. As estimated from the results given above, amount of phenolic compounds were less in hexane and dichloromethane extracts than the others. In conclusion, antioxidant potentials of polar and non-polar methanol extracts could be attributed to their high phenolic content.

Key words: Antioxidant activity, β-carotene/linoleic acid, DPPH, grape seeds, total phenolics

INTRODUCTION

Consumption of foods containing significant amounts of poly-unsaturated fatty acids has increased the importance and use of the antioxidants to prevent oxidation. The addition of antioxidants is a method of increasing the self-life, especially of lipids and lipid containing foods. Synthetic antioxidants, such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT), have restricted use in foods as these synthetic antioxidants are suspected to be carcinogenic (Madavi and Salunkhe, 1995). Therefore, the importance of search for natural antioxidants, especially of plant origin, has greatly increased in recent years (Jayaprakasha and Jaganmohan Rao, 2000).

Grapes are considered as the world's largest fruit crops, with an approximate annual production of 65 million metric tones. Functional ingredients of grape seeds include several flavonoids with a phenolic nature such as monomeric flavonoids (catechin and epicatechin), dimeric, trimeric and polymeric procyanidins and phenolic acids (gallic acid and ellagic acid). These flavonoids have been reported to exhibit antioxidant activity *in vivo* and *in vitro* in a number of studies. The antioxidant activity of flavonoids is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders (Yilmaz and Toledo, 2004).

Flavonoids easily scavenge aqueous free radicals (Teissedre and Landrault, 2000) because of their amphipatic characteristics (Riou *et al*., 2002). Polyvalent phenols in the flavonoid molecular structure allow some flavonoids to chelate metal ions. Phenolic acids, precursors of flavonoids, such as hydroxycinamic acid

Corresponding Author: Mustafa Kelen, Department of Horticulture, Faculty of Agriculture, Suleyman Demirel University, 32260 Isparta, Turkey

(cafeic, coumaric, ferulic and sinapic acids), hydroxycoumarin (scopoletin) and hydroxybenzoic acids (ellagic, gallic and vanillic acids) can form complexes with metals (Reische *et al*., 1998). Flavonoids can also scavenge superoxide anions (Robak and Gryglewski, 1988).

The literature outlines different approaches for determination of the antioxidant activities of the plant extracts. Therefore, different methodological approaches lead to scattered results, which are hardly comparable and often conflicting (Zygadlo *et al*., 1995; Mantle *et al*., 1998; Ruberto and Baratta, 2000; Koleva *et al*., 2002). A plethora of different antioxidant assays in available and because results rely on different mechanisms, they strictly depend on the oxidant/antioxidant models employed and on lipophilic/hydrophilic balance (Frankel *et al*., 1994). A single/substance/single-assay produces relative results and it is perceived as a reductive approach whenever a phyto-complex is involved. Therefore, antioxidant activities of the plant extracts studied here were determined by two complementary test systems namely DPPH free radical scavenging and β-carotene/linoleic acid systems.

The aim of present research is to determine *in vitro* antioxidant activities and amount of total phenolics of various extracts prepared by using solvents of varying polarity from three different seed of grape varieties (*Vitis vinifera* L.) of Turkish flora.

MATERIALS AND METHODS

Collection of plant material: Localities and collection periods of the varieties studied are as follow;

- Siyah Gemre: Deregumu village, Isparta, Turkey; 15th September, 2004 (Berry color: black)
- Adana Karasi: Duzici, Osmaniye, Turkey; 15th August, 2004 (Berry color: Black)
- Tilki Kuyrugu: Deregumu village, Isparta, Turkey; 15th September, 2004 (Berry color: White)

The voucher specimens have been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher No. 1-AA3435; 2-AA3419; 3-AA3436, respectively).

Preparation of the extracts: Extracts of ground grape seeds were prepared by using solvents of hexane, dichloromethane and methanol. A portion (100 g) of grape seeds was extracted with hexane (HE) (5.15, 6.91 and 18.15%; w/w, respectively), followed by dichloromethane (DCM) (1.82, 1.75 and 1.94%; w/w, respectively) and methanol (MeOH) in a Soxhlet apparatus (6 h for each solvent) (Sokmen *et al*., 1999). Methanolic extracts were also further fractionated to obtain polar (8.19, 5.27 and 13.14%; w/w, respectively) and non-polar sub-fractions (2.27, 1.51 and 3.18%; w/w, respectively).

Antioxidant activity

DPPH assay: The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay (Pharmacia, Uppsala, Sweden-LKB-Novaspec II) uses stable radical diphenylpicrylhydrazyl (DPPH) as a reagent (Sigma-Aldrich) (Cuendet *et al*., 1997; Burits and Bucar, 2000). Fifty microliter of various concentrations of the extracts in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I%) was calculated as follows:

$$I\% = (A_{blank}-A_{sample}/A_{blank})\times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% Inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration. Tests were carried out in triplicate and Butylated Hydroxytoluene (BHT), ascorbic acid, curcumine and α-tocopherol were also used as positive controls.

β-Carotene-linoleic acid assay: In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Barriere *et al*., 2001).

A stock solution of β-carotene/linoleic acid (Sigma-Aldrich) was prepared as follows. First, 0.5 mg of β-carotene was dissolved in 1 mL of chloroform (HPLC grade), then 25 μL of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated using a vacuum evaporator (Büchi, Flawil, Switzerland). Then 100 mL of distilled water saturated with oxygen (30 min at 100 mL min^{-1}) was added with vigorous shaking. Aliquots (2.5 mL) of this reaction mixture were transferred to test tubes and 350 μL portions of the extracts (2 mg mL^{-1} in ethanol) was added before incubating for 48 h at room temperature. The same procedure was repeated with BHT at the same

concentration and a blank containing only 350 μL of ethanol. After the incubation period the absorbances of the mixtures were measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHT and the blank.

Assay for total phenolics: Total phenolic constituents of aforesaid extracts of *S. tomentosa* were performed employing the literature methods involving Folin-Ciocalteu reagent and gallic acid as standard (Slinkard and Singleton, 1977; Chandler and Dodds, 1983). 0.1 mL of extract solution containing 1000 μg extract was taken in a volumetric flask, 46 mL distilled water and 1 mL Folin-Ciocalteu reagent were added and flask was shaken thoroughly. After 3 min, 3 mL of solution 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated to all standard gallic acid solutions (0-1000 mg 0.1 mL^{-1}) and standard curve was obtained that equation given below:

Absorbance: 0.0012×Gallic acid (μg) + 0.0033

RESULTS AND DISCUSSION

In the light of the differences among the wide number of test systems available, the results of a single-assay can give only a reductive suggestion of the antioxidant properties of extracts toward food matrices and must be interpreted with some caution. Moreover, the chemical complexity of extracts, often a mixture of dozens of compounds with different functional groups, polarity and chemical behavior, could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays in screening work is highly advisable. Among the plethora of methods that can be used for the evaluation of the antioxidant activity (TEAC, TRAP, LDL, DMPD, FRAP, ORAC, DPPH, PCL and β-carotene bleaching), very few of them (TEAC, DPPH, PCL) are useful for determining the activity of both hydrophilic and liphophilic species, thus ensuring a better comparison of the results and covering a wider range of possible applications (Sacchetti *et al*., 2005). Taking this into account, the *in vitro* antioxidant activity of the extracts tested, compared to that of BHT, ascorbic acid and curcumine and α-tocopherol were assessed by two different tests; DPPH free radical scavenging and β-carotene/linoleic acid systems.

Free radical scavenging capacities of the corresponding extracts were measured by DPPH assay and the results are shown in Table 1. In this system, polar subfraction of the methanolic extracts of grape seeds exhibited notable antioxidant potential than those of non-polar ones. The most active polar extract was Adana Karasi with an IC_{50} value of 5.90±0.20 μg mL^{-1}, while Siyah gemre exerted greater antioxidant activity than those of other non-polar extracts (7.00±0.40 μg mL^{-1}). All of the polar and non-polar extracts obtained from grape seeds studied here exhibited greater antioxidant activities than that of synthetic antioxidant BHT (18.00±0.40 μg mL^{-1}). On the other hand, hexane and dichloromethane extracts were remained almost inactive.

In β-carotene/linoleic acid assay, similar activity patterns were obtained (Table 2). As happened in DPPH system, polar extracts were also exerted greater activities than those of non-polar ones in this system. Especially, inhibition ratio of the oxidation of linoleic acid of the polar sub-fraction of Siyah gemre (95.62±3.54%) was too close to the synthetic antioxidant BHT (96.60±1.29%).

Based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents as described above, results of the colorimetric

Table 1: Free radical scavenging capacities of the extracts measured in DPPH assay[1]

Varieties	Extracts	IC_{50} (μg mL^{-1})
Siyah gemre	Hexane	-
	Dichloromethane	-
	Methanol/polar sub-fraction	6.90±0.40
	Methanol/non-polar sub-fraction	7.00±0.40
Adana karasi	Hexane	-
	Dichloromethane	-
	Methanol/polar sub-fraction	5.90±0.20
	Methanol/non-polar sub-fraction	11.00±0.30
Tilki kuyrugu	Hexane	-
	Dichloromethane	-
	Methanol/polar sub-fraction	6.40±0.50
	Methanol/non-polar sub-fraction	12.60±0.70
	BHT	18.00±0.40
	Ascorbic acid	3.80±0.10
	Curcumine	7.80±0.30
	α-tocopherol	6.50±0.70

[1]Results are means of three different experiments

Table 2: Inhibition ratio of the linoleic acid oxidation by the extracts[1]

Varieties	Extracts	Inhibition (%)
Siyah gemre	Hexane	-
	Dichloromethane	-
	Methanol/polar sub-fraction	95.62±3.54
	Methanol/non-polar sub-fraction	87.03±2.49
Adana karasi	Hexane	-
	Dichloromethane	-
	Methanol/polar sub-fraction	88.04±2.42
	Methanol/non-polar sub-fraction	83.60±1.57
Tilki kuyrugu	Hexane	-
	Dichloromethane	-
	Methanol/polar sub-fraction	80.60±1.13
	Methanol/non-polar sub-fraction	59.94±1.74
	BHT	96.60±1.29
	Ascorbic acid	94.50±2.14
	Curcumine	89.30±1.86
	α-tocopherol	96.65±1.72

[1]Results are means of three different experiments

Table 3: Amounts of total phenolic compounds in grape seed extracts[1]

Varieties	Extracts	Gallic acid equivalent (μg mg^{-1})
Siyah gemre	Hexane	11.94±1.54
	Dichloromethane	14.92±1.26
	Methanol/polar sub-fraction	197.33±3.52
	Methanol/non-polar sub-fraction	199.55±2.72
Adana karasi	Hexane	8.42±1.44
	Dichloromethane	16.77±1.62
	Methanol/polar sub-fraction	181.76±2.64
	Methanol/non-polar sub-fraction	195.72±4.17
Tilki kuyrugu	Hexane	6.44±1.74
	Dichloromethane	11.75±1.49
	Methanol/polar sub-fraction	187.65±3.43
	Methanol/non-polar sub-fraction	196.92±1.76

[1]Results are means of three different experiments

analysis of total phenolics are given in Table 3. The amount of the total phenolics was highest in the polar sub-fractions, followed by non-polar sub-fractions of grape seeds. The lowest amount of total phenolics was recorded in hexane extracts of the seeds. It is extremely important to point out that, there is a positive correlation between antioxidant activity potential and amount of phenolic compounds. Additionally, in grape seeds, no relation was found between antioxidant activities and total phenolic compounds and the color of berries that the seeds were taken from.

The presence of bioactive compounds in grapes, mainly phenolic compounds and the synergistic effects among them, have been related to their antioxidative properties (Frankel *et al*., 1995; Soleas *et al*., 1997). The phenolic compounds in grapes range from simple compounds (monomers) to complex tannin-type substances (oligomers and polymers). The antioxidant compounds present in grape have been identified as phenolic acids (benzoic and hydroxycinnamic acids), stilbene derivatives (resveratrol), flavan-3-ols (catechin, epicatechin), flavonols (kaempferol, quercetin, myrcetin) and anthocyanins (Miller and Rice-Evans, 1995; Vinson and Hontz, 1995; Ghiselli *et al*., 1998). One of the most abundant of these phenolic compounds is the flavan 3-ol, catechin (Singleton, 1988). The flavan 3-ols are mainly localized in the seeds and the skin (Thorngate and Singleton, 1997) although traces of monomers and dimers have been detected in the pulp (Bourzeix *et al*., 1986; Ricaroo-Da-Silva *et al*., 1992). The phenolic acids of grape are hydrxycinnamic acids which are in the form of esters of the tartaric acid in the skin and pulp (Ribereau-Gayon, 1965). On the other hand, the flavonols present in the white and red grape are localized only in the skin (Wulf and Nagel, 1980; Cheynier and Rigaud, 1986). Similarly, the stilbene derivatives are only located in the skin of the grapes (Jeandet *et al*., 1991; Lamuela-Raventos *et al*., 1995). The anthocyanidins, present only in red grapes, are generally localized in the skin (Amrani-Joutei, 1993) and for some type of vines, in pulp (Pecket and Small, 1980). The procyanidin composition of grape seeds has been determined (Lee and Jarowski, 1997). Escribano-Bailon *et al*. (1992) has reported 17 chemical constituents in *Vitis vinifera* grape seeds. Gabetta *et al*. (2000) reported the presence of monomers to heptamers and their gallates in grape seeds.

In order to prolong the storage stability of foods and to reduce the damage to human body, synthetic antioxidants are used for industrial processing. But according to toxicologists and nutritionists, the side effects of some synthetic antioxidants such as Butylated Hydroxyanisole (BHU) and Butylated Hydroxytoluene (BHT) have already been documented. For example these substances can show carcinogenic effects in living organisms (Ames, 1983; Baardseth, 1989). From this point of view, governmental authorities and consumers are concerned about the safety of their food and about the potential effects of synthetic additives on health (Reische *et al*., 1998). When compared to the antioxidative potentials of the standard compounds used in this study (BHT, ascorbic acid, curcumine and α-tocopherol), methanolic sub-fractions of the grape seeds exerted strong antioxidant activity. Especially, antioxidative performance of the sub-fractions was greater than that of BHT. In conclusion results of our study provides evidence that methanolic extracts with their sub-fractions deal with here could be used in the field of natural products.

REFERENCES

Ames, B.M., 1983. Dietary carcinogens and anticarcinogens: oxygen radical and degenerative diseases. Science, 221: 1256-1263.

Amrani-Joutei, K., 1993. Localisation des anthocyanes et des tanins dans le raisin. Etude de leur extractibilite. Ph.D Thesis, University of Bordeaux II.

Baardseth, P., 1989. Effect of selected antioxidants on the stability of dehydrated mashed potatoes. Food Additives and Contaminants, 6: 201-207.

Barriere, C., D. Centeno, A. Lebert, S. Leroy-Setrin, J.L. Berdague and R. Talon, 2001. Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. FEMS Microbiol. Lett., 201: 181-185.

Bourzeix, M., N. Weyland, D. Hereidia and C. Desfeux, 1986. Etude des catechines et des procyanidols de la grappe de raisin, du raisin et d'autres derives de la vigne. Bulletin de IOIV, 59: 669-670.

Burits, M. and F. Bucar, 2000. Antioxidant activity of Nigella sativa essential oil. Phytotheraphy Res., 14: 323-328.

Chandler, S.F. and J.H. Dodds, 1983. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum lacinitum*. Plant Cell Rep., 2: 105.

Cheynier, V. and J. Rigaud, 1986. HPLC separation and characterisation of flavonols in the skin of *Vitis vinifera* var. cinsault. Am. J. Enol. Viticulture, 37: 248-252.

Cuendet, M., K. Hostettmann and O. Potterat, 1997. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helvetica Chimica Acta, 80: 1144-1152.

Escribano-Bailon, T., Y. Gutierrez-Fernandez, J.C. Rivas-Gonzalo and C. Santos-Buelga, 1992. Characterisation of procyanidins of *Vitis vinifera* variety tintal del pais grape seeds. J. Agric. Food Chem., 40: 1794-1799.

Frankel, E.N., S.W. Huang, J. Kanner and J.B. German, 1994. Interfacial phenomena in the evaluation of antioxidants: Bulk oils versus emulsions. J. Agric. Food Chem., 42: 1054-1059.

Frankel, E.N., A.l. Waterhouse and P.L. Teissedre, 1995. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoprotein. J. Agric. Food Chem., 43: 890-894.

Gabetta, B., N. Fuzzati, A. Griffini, E. Lolla, R. Pace, T. Ruffilli and F. Peterlongo, 2000. Characterisation of proanthocyanidins from grape seeds. Fitoterapia, 71: 162-175.

Ghiselli, A., M. Nardini, A. Baldi and C. Scaccini, 1998. Antioxidant activity of different phenolic fractions separated from an Italian red wine. J. Agric. Food Chemistry, 46: 361-367.

Jayaprakasha, G.K. and L. Jaganmohan Rao, 2000. Phenolic constituents from lichen *Parmotrema stuppeum* (Nyl.) Hale and their antioxidant activity. Zeitschrift Für Naturforscung, 55c: 1018-1022.

Jeandet, P., R. Bessis and B. Gautheron, 1991. The production of resveratrol (3,5,4-trihydroxystilbene) by grape berries in different development stages. Am. J. Enol. Viticulture, 42: 41-46.

Koleva, I.I., van T.A. Beek, J.P.H. Linssen, A. de Groot and L.N. Evstatieva, 2002. Screening plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem. Anal., 13: 8-17.

Lamuela-Raventos, R.M., A.I. Romero-Perez, A.L. Waterhouse and M.C. de la Torre-Boronat, 1995. Direct HPLC analysis of cis-and trans-resveratrol and picied isomers in Spanish red *Vitis vinifera* wines. J. Agric. Food Chem., 43: 281-283.

Lee, C.Y. and A.W. Jarowski, 1997. Phenolic compounds in white grapes grown in New York. Am. J. Enol. Viticulture, 38: 277.

Madavi, D.L. and D.K. Salunkhe, 1995. Toxicological Aspects of Foods Antioxidants. In: Food Antioxidants. Madavi, D.L., S.S. Deshpande and D.K. Salunkhe (Eds.). New York: Marcel Dekker Inc, pp: 267.

Mantle, D., J.G. Anderton, G. Falkous, M. Barnes, P. Jones and E.K. Perry, 1998. Comparison of methods for determination of total antioxidant status: Application to analysis of medicinal plant essential oils. Comparative Biochem. Physiol. Part B, 121: 385-391.

Miller, N.J. and C.A. Rice-Evans, 1995. Antioxidant activity of resveratrol in red wine. Clin. Chem., 41: 1789-1792.

Pecket, R.C. and C.J. Small, 1980. Occurrence, location and development of anthocyanoplasts. Phytochemistry, 19: 2571-2576.

Reische, D.W., D.A. Lillard and R.R. Eitenmiller, 1998. Antioxidants. In: Food Lipids: Akoh, C.C. and D.M. Min (Eds.), Chemistry, Nutrition and Biotechnology. New York: Marcel Dekker Inc.

Ribereau-Gayon, P., 1965. Les acides-phenols de *Vitis vinifera*. Compted Rendus de I'Academic des Sciences de Paris, 260: 341-343.

Ricaroo-Da-Silva, J.M., J.P.H. Rosec, M. Bourzeix, J. Mourgues and M. Moutounet, 1992. Dimer and trimer procyanidins in Carignan and Mourvedre grapes and red wines. Vitis, 31: 55-63.

Riou, V., A. Vernhet, T. Doco and M. Moutounet, 2002. Aggregation of grape seed tannins in model wine-effect of wine polysaccharides. Food Hyrdrolloids, 16: 17-23.

Robak, J. and R.J. Gryglewski, 1988. Flavonoids are scavengers of superoxide anions. Biochem. Pharmacol., 37: 837-841.

Ruberto, G. and M.T. Baratta, 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem., 69: 167-174.

Sacchetti, G., S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice and R. Bruni, 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem., 91: 621-632.

Singleton, V.L., 1988. Wine Phenol. In: Modern Methods of Plant Analysis. Linskens, H.F. (Ed.), Vol. 4. Berlin: Springer-Verlag.

Slinkard, K. and V.L. Singleton, 1977. Total phenol analyses: Automation and comparison with manual methods. Am. J. Enol. Vitic., 28: 49-55.

Sokmen, A., B.M. Jones and M. Erturk, 1999. The *in vitro* antibacterial activity of Turkish plants. J. Ethnopharmacol., 67: 79-86.

Soleas, G.P., E.P. Diamendis and D.M. Goldberg, 1997. Wine as a biological fluid: History, production and role in disease prevention. J. Clin. Laboratory Anal., 11: 287-313.

Teissedre, P. and N. Landrault, 2000. Wine phenolics: Contribution to dietary intake and bioavailability. Food Res. Intl., 33: 461-467.

Thorngate, J.H. and V.L. Singleton, 1997. Localization procyanidins in grape seeds. Am. J. Enol. Viticulture, 45: 259-262.

Vinson, J.A. and B.A. Hontz, 1995. Phenol antioxidant index: Comparative antioxidant effectiveness of red and white wines. J. Agric. Food Chem., 43: 401-403.

Wulf, L.W. and C.W. Nagel, 1980. Identification and changes of flavonoids in Merlot and Cabernet *Sauvignon wines*. J. Food Sci., 45: 479-484.

Yilmaz, Y. and R.T. Toledo, 2004. Health aspects of functional grape constituents. Trends in Food Sci. Technol., 15: 422-433.

Zygadlo, J.A., A.L. Lamarque, D.M. Maestri and N.R. Grosso, 1995. Use of essential oils as natural antioxidants. Grasasy Aceites, 46: 285-288.