Extraction and Mixing Effects of Grape (Campbell) Seed Oil

Han-Chul Kang*, Young-Kyoo Min[1], Jong-Taek Hwang, Si-Dong Kim and Tae-Su Kim

Grape Experiment Station, Chungbuk Institute of Agricultural Technology, Okcheon 373-880,
[1]Department of Food Science and Technology, Chungbuk National University, Cheongju 361-763, Korea

Received August 3, 1999

Grape seed oil was extracted using different preparatory treatments as follows: (1) grinding, (2) grinding and roasting, (3) grinding and wet- roasting, (4) grinding, roasting, and wet-roasting, and (5) grinding, wet-roasting, and wet-roasting. The highest antioxidant activity was obtained from the sample with the method (2). Initial states of oxidation were similar except method (1) that showed more oxidized state, being P.O.V. 8. Acid values were observed in the range from 1.42 to 1.89. The lowest acid value was found as 1.42 in method (1) and those of others were somewhat higher, indicating that heating process of roasting produced some free fatty acids. From the results of sensory evaluation, the best odor and taste were obtained from the methods (2) and (3). Repetitive procedure of wet-roasting, like method 5, caused some loss of flavor components and decrease in the sensory evaluation score. Addition of grape seed oil (method 2) to soybean and perilla oil at the level of 20% retained considerable antioxidant activities as much as 4.3 and 5 times, respectively, than 100% soybean or perilla oil stored for 12 weeks. When soybean or perilla oil was mixed with 20% grape seed oils, P.O.V. decreased to half of that of unmixed oils.

Key words: *grape seed oil, extraction, antioxidation.*

Introduction

Grape has been for a long time consumed as a favorite food and raw material for food industry including wine production. However, grape seed oil is relatively new, produced only in few countries. As such, most of the seeds are not utilized. Nutritional composition of grape seed including protein, carbohydrate, and mineral constituents was determined, and physical and chemical characteristics of the oil were analyzed.[1,2] Grape seed oil, in particular, appears to be an excellent source of linoleic acid, showing 60-75% of total free fatty acid.[1,2]

Plant oil shows various chemical reactions such as polymerization, hydrolysis, and oxidation during storage or heating process.[3-9] Though synthetic antioxidants such as butylated hydroxyanisole and hydroxy-toluene are widely used as food additives, side effects in human are of concerns.[10] In addition, these antioxidants show other disadvantages of hydrolysis or volatility under high temperature, resulting in less efficacy of antioxidant activity.[11,12] It has been known that grape seed contains high concentrations of polyphenols, α-tocopherol, cathechin, and proanthocyanin, which function as antioxidant substances, and grape seed oil was suggested as an excellent plant oil.[1,13,14] Antioxidant activity and peroxide value of grape seed oil were compared with those of other plant oil such as sesame, perilla, soybean, and corn oil. The grape seed oil showed higher antioxidant activity than any other oil.[13]

Plant oil can be extracted through various unit operations such as pulvurization, heating, wet or dry-rendering, mechanical expression, continuous or filter press, solvent extraction, or some combinations of these processes. These different processes influence much or less recovery and factors of oil quality including antioxidant substances. In spite of excellent nutritional qualities, less investigation on the antioxidant substances has been performed in grape seed oil due to limited utilization. It is interesting to compare the qualities of various grape seed oil extracted by different preparatory treatments such as combinations of grinding, roasting, and wet-roasting. We report here on the quality comparison of grape seed oil extracted with different methods and mixing effects of combinations of different oil.

Materials and Methods

Plant materials. Grape vines of 5 years old vitis (Campbell early) were cultivated in a green house at temperatures ranging from 20 to 30°C. Three months after fruit-bearing, the ripe grapes were harvested. Grape seeds were then collected, washed, and stored at -20°C until use. The other plant sources such as perillas and beans were from Kangwon-do.

Extraction of seed oils. The grape seeds were partially dried for 5 days in the shade at room temperature. The seeds

*Corresponding author
Phone: 82-431-220-5363; Fax: 82-431-220-5365
E-mail: oksipo@chollian.net

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; P.O.V., peroxide value.

(each 1 kg) were fully ground using sample mill (Cyclotec, Sweden), and then treated with 5 different preparatory procedures as follows before extraction of oil: 1) simple grinding; no further treatment. 2) grinding and roasting; ground seeds were roasted for 15 min at 90°C. 3) grinding and wet-roasting; ground seeds were roasted with addition of water (100 ml; i.e., 10% of total volume(w)). 4) procedure 2 and wet roasting; seed samples were treated just as 2 and roasted with addition of 100 ml water. 5) procedure 3 and wet-roasting; samples were treated just as 3 and roasted with addition of 100 ml water. After these 5 different treatments, grape seed oil was extracted using oil compressor (Hanil, Korea). Soybean and perilla were treated with the same method as sample 2, and then oils were extracted using oil compressor.

On the other hand, grape seed oil was extracted using organic solvents. Grape seed (1 kg) was pulverized and mixed with 2 L chloroform/methanol (2:1) and N-hexane, respectively, and then agitated vigorously using motor stirrer (Chang shin, Korea) for 20 min. Sludges were eliminated through filter paper (Advantec, No. 6, Toyo). Grape seed oil from the resultant supernants were obtained by slow evaporation with rotary vacuum evaporator (Bibby, RE 100) at 50°C under almost atmospheric pressure (i.e., a little pressure) to minimize eventual loss of antioxidant substances.

Analytical methods. Antioxidant activity was routinely tested by measuring the electron donating ability of sample to 1,1-diphenyl-2- picrylhydrazyl (DPPH) according to the method of Blios.[15] The oil samples (30 μl) were introduced into the reaction mixture (total volume 1 ml) consisting of 100 μM DPPH, 50% ethanol, and Tris/HCl (pH 7.0, 20 mM). The reaction was carried out at 37°C for 30 min, and a decrease in the absorbance at 516 nm were measured spectrophotometrically. The decrease in absorbance from the control test was calculated as a reduction of DPPH (%) for the scavenging activity assessment. The control test was performed without oil sample.

Electron donating ability (%) = (1-A/B)×100
A: absorbance of test sample
B: absorbance of control

Oxidation state was determined by measuring P.O.V according to American Oil Chemist's Society method.[16] In brief, 1 ml test sample was mixed with 3 ml of CH_3COOH/$CHCl_3$ (3:2), then with 0.5 ml of saturated KI. After heating for 3 min, 5 ml of distilled water was added. The reaction mixture was titrated with 0.01 N sodium thiosulfate after the addition of 0.5 ml starch solution.

To investigate the additive effect, grape seed oil was added to soybean and perilla oil at different levels. These oil mixtures were tested for antioxidant activity after different times of preservation at room temperature under dark condition. The oxidation state of mixed oils was also measured by P.O.V. test. All experiments relating to the antioxidant activity and P.O.V. were performed in duplicates and mean values were calculated.

Alternatively, antioxidant substances were indirectly quantitated using phosphomolybdic acid preparation (Alltech, U.S.A.) according to manufacturer's instruction with minor modification. In brief, grape seed oil (50 μl) was mixed with phosphomolybdic acid reagent (500 μl), and boiled at 100°C for 5 min. Relative amount of blue color produced was compared by measuring Abs_{700nm} using spectrophotometer (Milton Roy 3000) after addition of 1 ml chloroform/methanol (1:1).

Total phenolic compound. Content of total soluble phenolic compounds was measured according to the method of Swain and Hills.[17] Distilled water 7.5 ml and extracted oil 100 μl were mixed. One half ml of 0.2 N Folin-ciocalteu reagent (sigma) and 500 μl saturated sodium carbonate solution were added, and final reaction volume was adjusted to 10 ml using distilled water. After 30 min of incubation at room temperature, absorbance at 760 nm was measured with the spectrophotometer. Content of total phenolic compound was represented as amount equivalent to gallic acid.

Sensory test. In an attempt to evaluate the sensory quality of grape seed oil extracted using different preparatory treatments, olfactory test was performed on the oil with 20 panels (equal number of men and women), and respective odors were evaluated. The sensory evaluation was graded into 5 classes as follows: 10, very good; 8, good; 6, moderate; 4, bad; 2, very unpleasant. The resultant scores were expressed as average values.

Results and Discussion

Grape seed oil samples were extracted through different preparatory treatments. Though recovery yields of crude oil samples were similar despite different treatments, sample (1) showed the highest value, 14.3%. Other samples showed only a little lowered extents with similar quantities, ranging from 12.9 to 13.8% (Table 1). Initial state of oxidation was evaluated by determining peroxide value after 24 h of oil extraction. The sample 3 showed the least oxidation state, resulting in 6 meq/kg·oil and sample 1 the highest value, 8 meq/kg·oil. Heat treatment (samples 2, 3, 4, and 5) resulted in a less oxidation state than the non-treated sample. Overally, however, the initial peroxide values were similar,

Table 1. Recovery and chemical characteristics of grape seed oil extracted through different methods.

Extraction method	Recovery (%)	P.O.V. (meq/kgoil)	Acid value
(1) Grinding	14.3	8	1.42
(2) Grinding and roasting	13.7	7	1.82
(3) Grinding and wet-roasting	13.8	6	1.79
(4) (2) and wet-roasting	13.7	7	1.88
(5) (3) and wet-roasting	12.9	7	1.89

The oxidation states of grape seed oils were expressed as P.O.V.

Table 2. DPPH scavenging activity and content of antioxidant substances.

Extraction method	DPPH scavenging activity (%)	Abs_{700nm} tested by phosphomolybdic acid	Phenolic compound (mg/ml)
(1) Grinding	63	0.149	16.4
(2) Grinding and roasting	86	0.205	24.2
(3) Grinding and wet-roasting	79	0.213	22.4
(4) (2) and wet-roasting	81	0.190	20.5
(5) (3) and wet-roasting	74	0.167	19.9
Chloroform/methanol	87	0.214	22.6
N-Hexane	79	0.195	19.8

The initial activities of antioxidant were tested for the grape seed oils extracted through different methods. The activity was expressed as the reduction (%) of DPPH from control value. The control test was performed without oil sample.

ranging from 6 to 8, in spite of preparatory treatments. Changes in these oxidation states can be correlated with the antioxidant activities but oxidation states of oil samples were similar at initial stage.. The acid values were tested for the oil samples. Even though the extent of difference is low, production of free fatty acids was the least for sample 1, showing 1.42 acid value. The values ranged from 1.42 to 1.89, indicating that free fatty acids were produced at a low level and not affected considerably by different preparatory treatments.

Since grape seed oil contains various antioxidant substances including α-tocopherol and (+)cathechin, initial antioxidant activities of the grape seed oil samples were represented as total scavenging activity by measuring DPPH reduction (Table 2). Among the 5 samples (1-5), the decrease in absorbance at 516nm was the highest for sample 2, resulting in 86% of DPPH reduction. Sample 1 was prepared through grinding only without heating process, and the antioxidant activity was the lowest, showing 63% of DPPH reduction. DPPH reductions of other samples (3, 4, and 5) were similarly ranged between 74 to 81%. The oil samples extracted through organic solvents appears to liberate more antioxidant substances, resulting in 79-87% of DPPH reduction. The content of antioxidant substances was analyzed by phosphomolybdic acid test and total phenolic compounds. The oil extracted using chloroform/methanol showed the highest Abs_{700nm} 0.214, suggesting that this fraction contains the most abundant reducing compounds. On the other hand, the content of phenolic compound was the highest from sample 2, showing 24.2 mg/ml and the chloroform/methanol fraction secondly 22.6 mg/ml. These results suggest that antioxidant substances can be more liberated from grape seed matrix *via* chemical or physical treatment. As such, simple grinding method is not recommendable for the liberation of antioxidant substances in the grape seed oil. Considering together, the grinding plus roasting method (sample 2) was the best for the production of antioxidant substances during the grape seed oil extraction. Maillard products during oil process can increase antioxidant activity.[18] However, the increase of antioxidant activity in the grape seed oil due to heat treatment appears to be more correlated with liberation of antioxidants rather than maillard products. Because reducing and phenolic compounds increased through proper heat treatment. In addition, heat treatment, 90°C 15 min in this experiment, is not sufficient to occur maillard reaction.

Table 3. Sensory evaluation of grape seed oils extracted by different methods.

Extraction method	Odor	Taste
(1) Grinding	6.5	7.1
(2) Grinding and roasting	9.2	9.3
(3) Grinding and wet-roasting	9.1	9.5
(4) (2) and wet-roasting	8.5	9.0
(5) (3) and wet-roasting	7.4	7.5

10, very good; 8, good; 6, moderate; 4, bad; 2, very unpleasant.

The odor and taste of oil samples were evaluated through sensory test by 20 panels (Table 3). They were more discriminated according to different preparatory treatments. Odor was most pleasant in sample 2 followed by 3, 4, 5, and 1. The best taste was obtained in sample 3 followed 2, 4, 5, and 1. In all, oil sample 1 extracted after simple grinding obtained the lowest score of evaluation. The most satisfactory overall quality of oil was observed in samples 2 and 3. These results coincide with the general concept that some flavoring sources are produced more by heating process. Despite preparatory heating treatments, repetitive roasting with water (sample 5) deteriorates the quality of grape seed oil as shown in sensory evaluation somewhat lowered.

In an attempt to increase antioxidant activity of complex oils, the grape oil sample 2 was mixed with soybean and perilla oils that were extracted by the same method as the sample 2. With these samples, antioxidant activity and oxidation state were determined up to 12 weeks. Entire soybean oil showed 25% of DPPH reduction at the initial stage, and the activity decreased to 15% after 12 weeks of preservation (Fig. 1). But mixed oil consisting of soybean and grape seed oil (80:20) showed higher antioxidant activity at the initial stage, resulting in 78% DPPH reduction which corresponds to 3.1 times increase over 100% soybean oil. This mixed oil retaining 65% of the activity after 12 weeks showed about 4.3 times increase in antioxidant activity. Thus, the antioxidant activity of soybean oil rapidly increased by the addition of grape seed oil at the level of 20% and was retained considerably up to 12 weeks tested. A

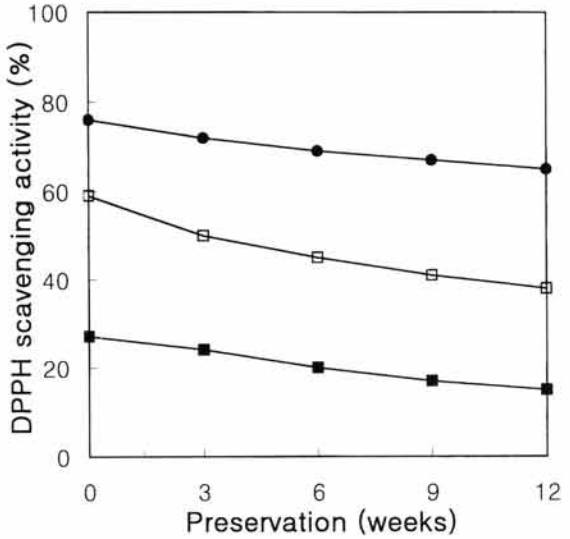

Fig. 1. Antioxidant activities of soybean and grape seed oil mixture. Soybean oil was mixed with grape seed oil by 0% (■), 10% (□), and 20% (●). The mixture was preserved at room temperature under light protection up to 12 weeks. Antioxidant activities was tested every 3 weeks.

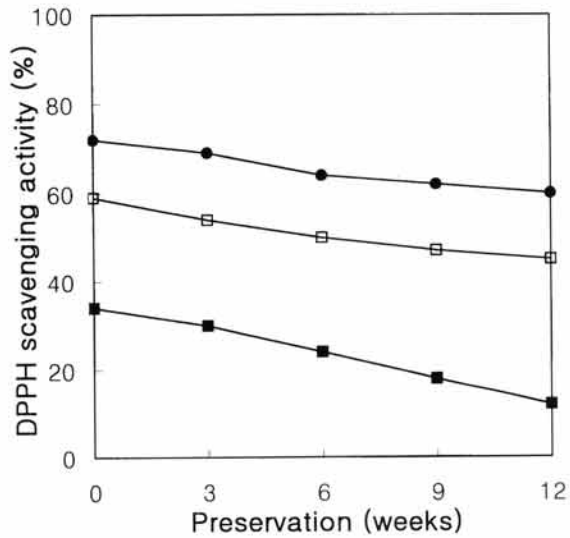

Fig. 2. Antioxidant activities of perilla and grape seed oil mixture. Perilla and grape seed oil were mixed through addition of grape seed oil by 0% (■), 10% (□), and 20% (●) to perilla oil. The resulting mixtures were stored at room temperature under light protection. The antioxidant activities were measured every 3 weeks up to 12 weeks.

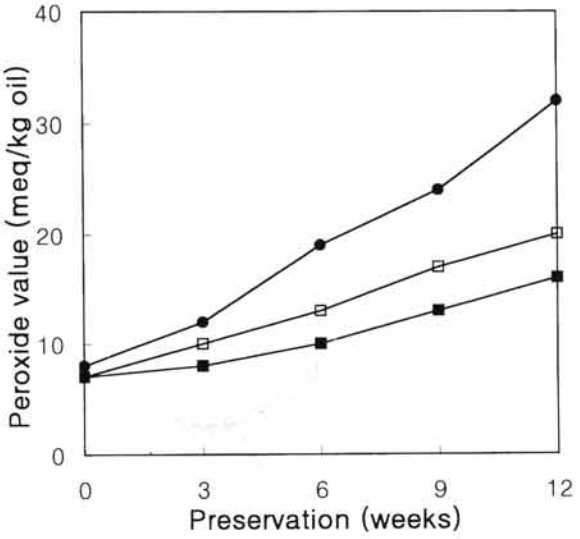

Fig. 3. Effect of grape seed oil on peroxide value of soybean and grape seed oil mixture. Soybean oil was mixed with grape seed oil by 0% (■), 10% (□), and 20% (●). These mixtures were preserved at room temperature under light protection up to 12 weeks. Peroxide values were measured every 3 weeks.

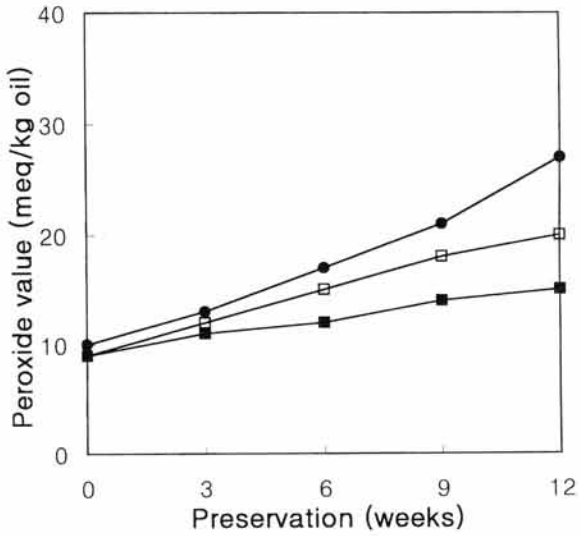

Fig. 4. Effect of grape seed oil on peroxide value of perilla and grape seed oil mixture. Perilla and grape seed oil were mixed by addition of grape seed oil by 0% (■), 10% (□), and 20% (●) to perilla oil. The resulting perilla and grape seed oil mixtures were stored at room temperature under light protection. Peroxide values were measured every 3 weeks up to 12 weeks.

similar result was observed in the mixture of perilla-grape seed oil (Fig. 2). Addition of only 20% of grape seed oil to perilla oil resulted in 2.2 times increase of antioxidant activity at the initial stage, and the activity was retained considerably during the preservation period. Mixture of 80% perilla oil and 20% grape seed oil resulted in five fold increase of activity in comparison with 100% perilla oil after 12 weeks storage. It appears that the decrease rate of antioxidant activity was more retarded by the addition of grape seed oil to perilla oil. The retardation of antioxidant activity, in both cases of soybean and perilla plus grape seed oil, suggests that antioxidant cofactors may exist more in the grape seed oil than in soybean and perilla oil. However, such synergetic cofactor was not confirmed in this experiment.

On the analogy of antioxidant activity ameliorated by grape seed oil, oxidation states of soybean and grape seed oil mixture was tested by measuring the peroxide values (Fig. 3). The initial peroxide values were similar between 100% soybean oil and mixed oils added with grape seed oil, but

differences increased proportionally to the preservation time. One hundred percent soybean oil showed P.O.V. 32, mixed oil with 20% grape seed oil 16, corresponding to two folds lowered value after 12 weeks of preservation. A similar result was observed in perilla and grape seed oil mixture. The mixture of 80% perilla and 20% grape seed oil showed 55% of P.O.V. in comparison with 100% perilla oil after the same preservation period (Fig. 4). In all cases, the initial P.O.V. of soybean or perilla mixed oils were low and similar level, being 7-8 and 9-10, respectively. However, the differences of P.O.V. between entire and mixed oils increased, and those of mixed oils were much retarded than 100% soybean or perilla oils. This phenomenon may be explicated by the increase of antioxidant activity due to grape seed oil.

Conclusions

The grinding and roasting method or grinding and wet-roasting method is recommendable as preparatory treatment for extraction of grape seed oil since production of antioxidant substances and sensory scores was ameliorated by such preparatory treatment. Addition of grape seed oil obtained through grinding and roasting to soybean or perilla oils increased rapidly the antioxidant activity of these mixed oils, and the activities were retained considerably for 12 weeks. Peroxide values of soybean and perilla oils were retarded more by addition of some grape seed oil. Thus, methods 2 and 3 can be proposed as proper extraction of grape seed oil. Soybean and perilla oils are consumed as polular plant oils in Korea, but these oils lack antioxidant activity. Thus, in view of supplementing mutually merits and desmerits, combinative utilization of grape seed oil with other plant oils may improve the excellent antioxidant activity and sensory scores.

References

1. Kamel, B. S. and Dawson, H. (1985) Characteristics and composition of melon and grape seed oils and cakes. *J. Am. Oil Chem. Soc.* **62**, 881-883.
2. Fedeli, E. (1983) Miscellaneous exotic oils. *J. Am. Oil Chem. Soc.* **60**, 404-406.
3. Fukuda, Y., Nagata, M., Osawa, T. and Namiki, M. (1986) Chamical aspects of the antioxidative activity of roasted sesame seed oil and the effect of using the oil for frying. *Agric. Biol. Chem.* **50**, 857-862.
4. Manley, C. H., Vallon, P. P. and Erickson, R. E. (1974) Some aroma components of roasted sesame seed (*Sesamum indicum* L.). *J. Food Sci.* **39**, 73-76.
5. Sleeter, R. T. (1981) Effects of processing on quality of soybean oil. *J. Am. Oil Chem. Soc.* **58**, 239-244.
6. Nawar, W. W. (1985) Chemistry of thermal oxidation of lipids. In *Flavor Chemistry of Fats and Oils*, Min, D. B. and Smouse, T. H. (eds.) pp. 39-47, American Oil Chemists' Society, Champaign, IL.
7. Nawar, W.W. (1985) Lipids. In *Food Chemistry*, Fennema, O. R. (2nd ed.) pp. 139-148, New York.
8. Richardson, T. and Finley, J. W. (1985) Chemical changes in food during processing, pp. 410, AVI publishing Company, Inc., Westport, Connecticut.
9. Yoon, S. H., Kim, S. K, Kim, K. H. Kwon, T. W. and Teah, Y. K. (1987) Evaluation of physicochemical changes in cooking oil during heating. *J. Am. Oil Chem. Soc.* **64**, 870-873.
10. Branen, A. L. (1975) Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J. Am. oil Chem Soc.* **52**, 59-63.
11. Jung, M. Y. and Rhee, K. C. (1994) Quality improvement and extension of useful life of frying cotton seed oil by adsorbent or chemical treatment. *Foods Biotech.* **3**, 65-70.
12. Peled, M., Gutfinger, T. and Letan, A. (1975) Effects of water and BHT on stability of cotton seed oil during frying. *J. Sci. Foods. Agric.* **26**, 1655-1659.
13. Kang, H. C., Park, W. J., Kim, S. D. and Park, J. C. (1998) Characterization of grape seed oil. *Agric. Chem. Biotechnol.* **41**, 578-582.
14. Fantozzi, P. (1981) Grape seed: A potential source of protein. *J. Am. Oil Chem. Soc.* **58**, 1027-1031.
15. Blios, M. S. (1958) Antioxidant determination by the use of a stable free radical. *Nature* **181**, 1199.
16. A.O.C.S. (1990) Official methods and recommended practices of the American Oil Chemist's Society (94th ed.), 8-53, American oil Chemist's Society, Champaign, IL.
17. Swain, T. and Hills, W. E. (1959) Phenolic constituents of *Ptunus domestica*. I. Quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* **10**, 63-68.
18. Larson, R. A. (1988) The antioxidants of higher plants. *Phytochemistry* **29**, 969-974.