Antioxidant Activity in Soybean Oil of Extracts from Thompson Grape Bagasse

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ABSTRACT: The phenolic compounds of Thompson grape bagasse were extracted using a 95:5 (vol/vol) ethanol/water mixture. Measurement of the antioxidant activity in refined soybean oil of bagasse grape extract was performed by using two different methods, the Rancimat method and the Schaal oven method in conjunction with peroxide value determination. The antioxidant activity of the extract was compared to the tertiary butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA) activity. The bagasse grape extract showed similar antioxidant activities in both methods employed. At all concentrations tested [0.1, 0.3 and 0.5% of total phenols (TP)] the extract exhibited appreciable activity, which exceeded the activity of BHA. At some concentrations (0.3 and 0.5% TP) the extract exhibited activity comparable to that of TBHQ. Bagasse is a by-product with a high content of phenolic compounds and is a good source of natural antioxidants.

Paper no. J9144 in *JAOCS 76,* 1445–1447 (December 1999).

KEY WORDS: Antioxidant activity, BHA, grape bagasse, phenolic, Rancimat, Schaal oven, soybean oil, TBHQ.

Antioxidant addition during industrial food formulations is one of the most effective means to retard fat oxidation. It is a popular method for increasing the shelf life of lipids and lipids-containing foods (1). Synthetic antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are widely used in many foods. However, their use has been questioned because of issues related to toxicity and carcinogenicity (2,3). For this reason, considerable attention has been given to the application of natural antioxidants in foods, because of their potential nutritional and therapeutic effects (1).

Flavonoids and other phenolic compounds have been reported to have multiple biological effects and antioxidant activities (4–6). Grapes are a major source of phenolic compounds among different fruits and vegetables (7). Phenolic compounds have been found at concentrations as high as 260–920 mg/kg in grapes and 1,800–3,200 mg/L in wines (6).

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Bourzeix (8) quantified the content and distribution of phenolic compounds in grape (Carignan noir variety), showing that 95% of these compounds are present in the bagasse. Presently, this by-product is discarded in Mexico. Therefore, the purpose of this study is to test the antioxidant activity of an extract obtained from the bagasse from Thompson seedless grapes, the main grape variety produced in Sonora, Mexico, as measured on the basis of the stability of refined soybean oil containing the extract.

EXPERIMENTAL PROCEDURE

Materials. Catechin, BHA, and TBHQ were obtained from Sigma Chemical Co. (St. Louis, MO). Ethanol and Folin-Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). All chemicals and solvents used throughout the present work were reagent grade.

Sample preparation. Green Thompson grapes, purchased directly from the local market, were mechanically pressed in the laboratory in order to collect the bagasse. The bagasse was then dried at room temperature until the moisture content reached 12% or less. Dried bagasse was ground in a Wiley laboratory mill (Arthur N. Thomas Co., Philadelphia, PA) to pass a mesh 30 sieve (600 μm), then stored at 4°C.

Alcoholic extraction. Phenolic compounds from the bagasse were extracted according to the procedure suggested by Onyencho and Hettiarachchy (9). The bagasse grape sample powder (10 g) was extracted with 50 mL of ethanol/water (95:5 vol/vol) during 2 h of continuous stirring using a wrist action shaker (Burrel model 75; Burrel Corporation, Pittsburgh, PA). The mixture was then centrifuged at 5000 rpm (2990 × *g*) in a Damon/IEC B-20A centrifuge (Damon IEC/Division, Needham Heights, MA). The supernatant was concentrated in a Yamato RE-47 rotatory evaporator (Yamato Scientific Co. Ltd., Tokyo, Japan) at 40–45°C under vacuum. Finally, the extract was dried at 60°C in a National 3640 vacuum oven (National Appliance Company, Portland, OR).

Analysis of phenolic compounds. Total phenols (TP) were assayed colorimetrically using the Folin-Ciocalteu method (10) as follows. An aliquot (1 mL) of the extract was mixed with 5.0 mL of 10-fold diluted Folin-Ciocalteu reagent and 15 mL 20% sodium carbonate solution. After 5 min at room

temperature, the absorbance was measured at 750 nm (PE UV-vis Lambda 2S; PerkinElmer de Mexico, Mexico City, Mexico). The content of phenolics was expressed as catechin equivalent (11).

Prooxidative elements quantification. Metallic compounds were analyzed in the bagasse extracts using the following procedure. The extracts (0.5 g) were digested with concentrated nitric and perchloric acids in a micro-Kjeldahl digestion apparatus (Labconco, Kansas City, MO). Aliquots were then taken for analysis. Phosphorus was analyzed using AOCS official method Ca 12-55 (12) with a spectrometer set at 650 nm (PE UV-vis Lambda 2S, PerkinElmer de Mexico). Copper and iron ions were determined by atomic absorption spectrometry (PE3100; PerkinElmer de Mexico). Wavelengths used for copper and iron were 324.7 and 248.3 nm, respectively. All experimental work was carried out in all-glass equipment to minimize metal contamination.

Antioxidant activity evaluation. Samples for the antioxidant activity evaluation were prepared by mixing the extract with the oil at three different concentrations of TP [0.1, 0.3, and 0.5% (w/w)] with the aid of monoglycerides as emulsifier. TBHQ and BHA at 0.02% (w/w) were used for comparison. Two methods were employed: the Rancimat method and the Schaal oven method in conjunction with peroxide value (PV) determination.

(i) Rancimat method. A model 679 Rancimat (Metrohm, Herisau, Switzerland) was used. A 3.0-g portion of each test sample was loaded into the reaction vessel. Measurements of three different samples were conducted in one batch. The air supply was maintained at 20 L/h and the heating temperature at 110 ºC throughout the experiment (13–15). Induction time for the test samples was determined by measuring the elapsed time from the beginning to the moment when a sudden change of conductivity occurred. The control was soybean oil with no additives.

(ii) Schaal oven method. A 50-g portion of each sample was placed in a glass jar and heated in a gravity convection oven set at 60 ± 1°C (16). The PV in the samples were measured in duplicate following the AOCS official method Cd 8-53 (12), at periods of 0, 7, 14, and 21 d.

Statistical analysis. All analytical values represent means of three replicates done on at least two different experiments. Data obtained were subjected to one-way analysis of variance and Tuckey's test analysis. Significance was declared at $P < 0.05$. Statgraphic software computer was used for statistical analysis (Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

Total phenolic contents. Total phenolic compound concentration in the green grape (Thompson variety) bagasse was 4.27 mg/g. The extract had a green-brown color, viscous texture, and iodine odor. Total phenolic content in the extract was 26.56 mg/g (Table 1). Larrauri *et al*. (17) reported higher levels of phenolic compounds for dried red grapes. According to Kanner *et al.* (6), red grape varieties have higher levels of phenolic compounds than green ones. Many solvents can successfully be used for the extraction of phenolic compounds; however, the extract yield increases as the polarity of the solvent increases (18). In this study, the concentration of phenolic compounds increased sixfold using ethanol.

TABLE 1
Total Phenolic Contents and Prooxidative Elements of Thompson Grape Bagasse and Extract[a]

	Bagasse	Bagasse extract
Total phenol[b] (mg/g)	4.27 ± 0.60[a]	26.56 ± 3.78[b]
Copper (ppm)	19.76 ± 1.09	ND
Iron (ppm)	101.40 ± 11.22[c]	25.25 ± 2.79[d]
Calcium (ppm)	45.59 ± 1.51[e]	39.55 ± 1.48[f]
Magnesium (ppm)	67.95 ± 10.38[g]	30.53 ± 4.66[h]
Phosphorus (ppm)	0.0042 ± 0.00	ND

[a]Values are mean ± SD ($n = 2$). Means within a row with different superscript roman letters (a–h) are significantly different ($P < 0.05$). ND, not detected.
[b]Catechin equivalents.

Prooxidative elements content. Some of the antioxidant activity of the phenolic compounds depends on the presence of metal catalysts such as copper (19). The levels of copper and iron in the bagasse were significantly higher ($P < 0.05$) than those found in the extract; however, copper was not detected in the processed extract (Table 1).

Antioxidant activity. The grape bagasse extract showed similar antioxidant activities when measured using both methods of analyses.

(i) Rancimat method. The induction times of the grape bagasse extract are shown in Table 2. At all concentrations tested (0.1, 0.3, and 0.5% of total phenols), the extract exhibited activity significantly higher than that of BHA ($P < 0.05$). The highest induction period was achieved with the extract at 0.5% TP; this activity exceeded significantly ($P < 0.05$) the antioxidant activity of both synthetic antioxidants.

(ii) Schaal oven method. The grape bagasse extract was tested for antioxidant activity in soybean oil at concentrations of 0.1 and 0.3% TP. Results are shown in Figure 1. At each test day, stronger antioxidant activities ($P < 0.05$) are indicated by lower PV with respect to the control. Although not significant, overall the antioxidant activity of grape bagasse extract was higher than that of BHA, but slightly lower than that of TBHQ.

TABLE 2
Antioxidant Activity (Rancimat test) of Thompson Grape Bagasse Extract and Synthetic Antioxidants[a]

Treatment	Induction time[b] (110°C)
Soybean oil (control)	6.24 ± 0.20[a]
Soybean oil + extract, 0.1% TP	15.20 ± 0.56[b]
Soybean oil + extract, 0.3 % TP	29.10 ± 1.84[c]
Soybean oil + extract, 0.5 % TP	>48.00[d]
Soybean oil + BHA, 0.02 % w/w	8.56 ± 0.37[e]
Soybean oil + TBHQ, 0.02 % w/w	21.70 ± 0.28[f]

[a]TP, total phenol; BHA, butylated hydroxyanisole; TBHQ, tertiary butyl hydroquinone.
[b]Values are mean + SD ($n = 2$). Means with different superscript letters (a–f) are significantly different ($P < 0.05$).

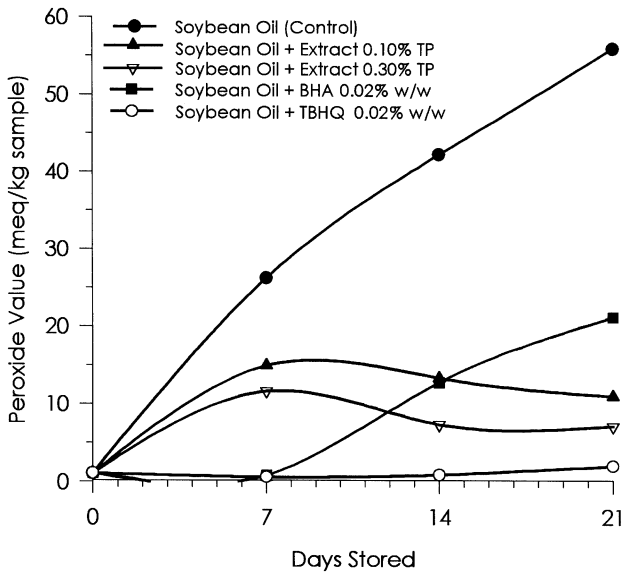

FIG. 1. Peroxide values of soybean oil with added ethanolic antioxidant extracts from Thompson grape bagasse during storage at 60 ± 1°C (Schaal test). TP, total phenol; BHA, butylated hydroxyanisole; TBHQ, tertiary butyl hydroquinone.

This work suggests that the ethanolic extracts from Thompson grape bagasses have a strong antioxidant activity. The bagasse, which is a by-product of the wine industry, has a high content of phenolic compounds that could be a good source of natural antioxidants.

ACKNOWLEDGMENTS

This work was supported by SIMAC (Sistema de Investigación del Mar de Cortés, Mexico; project no. SIMAC 94/DT-06) and by Industrias Vinicolas Pedro Domecq, S.A. de C.V. México.

REFERENCES

1. Halliwell, B., M.A. Murcia, S. Chirico, and O.I. Aruoma, Free Radicals and Antioxidants in Food and *in vivo*: What They Do and How They Work, *Crit. Rev. Food Sci. Nutr. 35*:7–20 (1995).
2. Branen, A.L., Toxicology and Biochemistry of Butylated Hydroxyanisole and Butylated Hydroxytoluene, *J. Am. Oil Chem. Soc*. *52*:29 (1975).
3. Imaida, K., S. Fukushima, T. Shivai, M. Ohtani, K. Nakanishi, and N. Ito, Promoting Activity of Butylated Hydroxyanisole and Butylated Hydroxytoluene on 2-Stage Urinary Bladder Carcinogenesis and Inhibition of Glutamyl Transpeptidase-Positive Foci Development in the Liver of Rats, *Carcinogenesis. 4*:895–899 (1983).
4. Hudson, B.J.F., and J.I. Lewis, Polyhydroxy Flavonoids Antioxidants for Edible Oils: Structural Criteria for Activity, *Food Chem. 10*:11–14 (1983).
5. Bors, W., and M. Saran, Radical Scavenging by Flavonoid Antioxidants, *Free Radical Res. Commun. 2*:131–134 (1987).
6. Kanner, J., E. Frankel, R. Granit, B. German, and J.E. Kinsella, Natural Antioxidants in Grapes and Wines, *J. Agric. Food Chem. 42*:64–69 (1994).
7. Macheix, J.J., A. Fleurient, and J. Billot, The Main Phenolics of Fruits, in *Fruit Phenolics*, CRC Press, Boca Raton, 1990, pp. 1–103.
8. Bourzeix, M., The Content and Distribution of Phenolic Compounds in Carignan Noir Grape, *C.R. Hebd. Seances Acad. Agric. Fr. 57*:197–203 (1970).
9. Onyeneho, S.N., and N.S. Hettiarachchy, Effect of Navy Bean Hull Extract on the Oxidative Stability of Soy and Sunflower Oils, *J. Agric. Food Chem. 39*:1701–1704 (1991).
10. Singleton, V.L., and J.A. Rossi, Colorimetry of Total Phenolics with Phosphomolybdic and Phosphotungstic Acid Reagents, *Am. J. Enol. Vitic. 16*:144–158 (1965).
11. Yi, O.S., A.S. Meyer, and E.N. Frankel, Antioxidant Activity of Grape Extracts in a Lecithin Liposome System, *J. Am. Oil Chem. Soc. 74*:1301–1307 (1997).
12. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* edited by D. Firestone, 4th edn., AOCS Press, Champaign, 1994.
13. Matthäus, B.W., Determination of Oxidative Stability of Vegetable Oils by Rancimat and Conductivity and Chemiluminescence Measurements, *J. Am. Oil Chem. Soc*. *73*:1039–1042 (1996).
14. Méndez, E., J. Sanhueza, H. Speisky, and A. Valenzuela, Validation of the Rancimat Test for the Assessment of the Relative Stability of Fish Oils, *Ibid*. *73*:1033–1036 (1996).
15. Medina-Juárez, L.A., P. Gonzalez-Díaz, N. Gámez-Meza, J. Ortega-García, A.B. Moreno-Varela, L. Bringas-Alvarado, and O. Angulo-Guerrero, Effects of Processing on the Oxidative Stability of Soybean Oil Produced in Mexico, *Ibid*. *75*:1729–1733. (1998).
16. Zhang, K.-Q., Y. Bao, P. Wu, R.T. Rosen, and C.-T. Ho, Antioxidative Components of Tanshen (*Salvia militiorrhiza* Bung), *J. Agric. Food Chem*. *38*:1194–1197 (1990).
17. Larrauri, J.A., P. Rupéres, and F. Saura-Calixto, Effect of Drying Temperature on the Stability of Polyphenols and Antioxidant Activity of Red Grape Pomace Peels, *Ibid. 45*:1390–1393 (1997).
18. Duh, P.-D., Antioxidant Activity of Burdock (*Arctium lappa* Linné): Its Scavenging Effect on Free-Radical and Active Oxygen, *J. Am. Oil Chem. Soc. 75*:455–461 (1998).
19. Huang, S.W., and E.N. Frankel, Antioxidant Activity of Tea Catechins in Different Lipid Systems, *J. Agric. Food Chem*. *45*:3033–3038. (1997).

[Received February 12, 1999; accepted July 20, 1999]