Canola Extract as an Alternative Natural Antioxidant for Canola Oil

Udaya N. Wanasundara[a] and **Fereidoon Shahidi**[a,b,*]
Departments of [a]Biochemistry and [b]Chemistry, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3X9 Canada

The antioxidative activity of ethanolic extracts of canola meal at 100, 200, 500 and 1000 ppm on refined-bleached (RB) canola oil was examined and compared with commonly used synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), BHA/BHT/monoglyceride citrate (MGC) and *tert*-butylhydroquinone (TBHQ). Stability of RB oil was monitored under Schaal oven test conditions at 65°C over a 17-d period. Progression of oxidation was monitored by weight gain, peroxide, conjugated diene, 2-thiobarbituric acid and total oxidation values. Canola extracts at 500 and 1000 ppm were more active than BHA, BHT and BHA/BHT/MGC and less effective than TBHQ at a level of 200 ppm.

KEY WORDS: Accelerated oxidation, antioxidant activity, canola extract, canola oil, natural antioxidants, oxidative stability, synthetic antioxidants.

Autoxidation is considered to be the main route of spoilage of edible oils, and its progression leads to oxidative rancidity *via* a free-radical chain mechanism (1). The unique fatty acid composition of canola oil differentiates it from other vegetable oils. Canola oil has a substantial amount (8–12%) of linolenic acid (C18:3) compared to other vegetable oils, such as soybean, sunflower, olive and corn, which contain 8.0, 0.2, 0.8 and 0.7%, respectively (2). The high content of unsaturated fatty acids, especially C18:3, in canola influences its stability and keeping quality. These unsaturated fatty acids undergo rapid autoxidation and produce undesirable off-flavors and off-odors during storage and heating (3). Therefore, it is necessary to stabilize canola oil to maintain its favorable qualities for food use.

Antioxidants are principal ingredients that protect food quality by retarding oxidative breakdown of lipids. Synthetic phenolic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ), are commonly used in fats and oils because of their effectiveness (1). However, their safety has been questioned (4). Therefore, synthetic food additives are being subjected to rigorous investigation by government agencies, and there is increasing pressure from consumer groups to reduce the amount of synthetic additives in foods (5). During the last few decades, natural alternatives for synthetic antioxidants have been studied. Antioxidative compounds from oilseeds, such as soybean, cottonseed, peanut and sesame, have been investigated by researchers (6–9).

Both the flour and methanolic extracts of mustard and canola seeds have been shown to possess strong antioxidative activities in meat model systems (10,11). Oil-free, dried canola meal contains 1–2% phenolic compounds, about ten times higher than those in soybean meal (12). Phenolic compounds of canola include phenolic acids, present in free, esterified or insoluble-bound forms (13), flavonoids (14) and condensed tannins (15). But, presence of high levels of these compounds in canola meal is undesirable because of their adverse effects on nutritional and sensory properties of the meal (12,16). Therefore, removal of these compounds from canola meal and their proper utilization would be a new development in the canola processing industries.

This study reports the effect of ethanolic extracts of canola meal on the oxidative stability of canola oil at elevated temperatures. The efficacy of this extract was compared with commonly used synthetic antioxidants such as BHA, BHT, TBHQ and a combination of BHA, BHT and monoglyceride citrate (MGC).

MATERIALS AND METHODS

Canola seeds and fresh refined-bleached (RB) canola oil, containing no antioxidants, were obtained from CSP Foods (Saskatoon, Saskatchewan, Canada). Synthetic antioxidants, namely TBHQ, BHA and BHT, were obtained from Sigma Chemical Company (St. Louis, MO). MGC was obtained from Griffith Laboratories (Scarborough, Ontario, Canada). The initial quality of the fresh canola oil was investigated by analysis for fatty acid composition (17), iodine and peroxide values (PV) (see Ref. 18, Method Cd 1-25).

Canola seeds were first ground in a Moulinex coffee grinder, then defatted with hexane in a Soxhlet apparatus and air-dried overnight. Defatted canola meal (6.0 g) was extracted with 100 mL 95% (vol/vol) ethanol for 20 min. This extraction was repeated two times, and the residual meal was separated by centrifugation. The resultant ethanolic extract were combined and evaporated to dryness under vacuum at 40°C. The dried canola extract (CE) at levels of 100, 200, 500 and 1000 ppm, TBHQ, BHA and BHT at 200 ppm and BHA/BHT/MGC at 100:100:50 ppm (MGC as citric acid equivalents) were applied to RB canola oil to examine their antioxidative activity. The dried extract and synthetic antioxidants were mixed with a minimum amount of absolute ethanol in an ultrasonic water bath and added to the oil (200 g) and again mixed for 10 min. A control sample contained only the same amount of ethanol used to dissolve additives. To observe the weight gain during the oxidation, the procedure of Olcott and Einset (19) was used with minor modifications. Each sample (2 g), prepared as mentioned above, was placed in a glass petri dish (60 mm × 15 mm), and traces of water were removed in a vacuum oven overnight at 35°C. The samples were reweighed and stored in a forced-air oven at 65°C. The rate of oxidation in terms of weight increase was recorded at 24-h intervals. Each sample (25 mL) was stored separately in a forced air oven at 65°C over a 17-d period in small open glass containers for other chemical analyses. Samples of each treatment were removed at 0, 2, 5, 9, 13 and 17-d intervals, flushed with nitrogen for 30 s, covered with aluminum foil, and stored at −20°C until further analyses were started (usually within a week).

Chemical analysis of the oils, subjected to accelerated oxidation, included determination of PV (see Ref. 18, Method Cd 8-53), conjugated dienes (CD) as shown by ultraviolet absorbance at 234 nm (see Ref. 20, Method 2.505), 2-thiobarbituric acid reactive substances (TBARS; see Ref. 18, Method Cd 19-90) and *p*-anisidine value (AnV;

*To whom correspondence should be addressed.

see Ref. 20, Method 2.504). The total oxidation (TOTOX) value was calculated as 2 PV + AnV (21).

All experiments and/or measurements were replicated three times. Mean values ± standard deviation were reported for each case. Analyses of variance and Tukey's studentized range test (22) were performed on the Statistical Analysis System (23) to evaluate the significant differences between different mean values.

RESULTS AND DISCUSSION

Fresh RB canola oil used in this study was of good initial quality with reasonably low PV and iodine values (Table 1). The fatty acid composition of oil indicated that it contained 57.7% oleic, 23.5% linoleic, 9.4% linolenic acids and a small amount of erucic acid (0.3%). Canadian standards require that high-quality canola oil has an iodine value between 110 and 126 g iodine/100 g oil, a PV below 2 meq/kg oil and erucic acid content less than 2% of its total fatty acids (24).

TABLE 1

Iodine and Peroxide Values and Fatty Acid Composition of Refined-Bleached Canola Oil (without additives)

Parameter	Content
Iodine value (g iodine/100 g oil)	112.0 ± 1.9
Peroxide value (meq/kg oil)	0.20 ± 0.01
Fatty acids (area %)	
C16:0	4.2 ± 0.0
C16:1	0.2 ± 0.0
C18:0	1.9 ± 0.0
C18:1	57.7 ± 0.1
C18:2	23.5 ± 0.1
C18:3	9.4 ± 0.0
C20:0	0.6 ± 0.0
C20:1	1.8 ± 0.0
C22:0	0.3 ± 0.0
C22:1	0.3 ± 0.0

Effect of CE on weight gain. The effect of added CE and synthetic antioxidants on weight gain of canola oil during accelerated oxidation is presented in Figure 1. The standard deviations for the weight gain data in all cases were less than ±0.01% and confined to the boundaries of the symbols used. The time required for a 0.5% weight increase (19) of oil sample, taken as the length of the induction period, was 4.0, 4.7, 5.0, 6.0, 6.3, 6.5, 7.8 and 8.5 d for oils containing BHA-200, BHT-200, CE-100, CE-200, BHA/BHT/MGC-250, CE-500, CE-1000 and TBHQ-200, respectively. The corresponding time for the control sample was 3.2 d. It has been suggested that each day under Schaal oven test conditions at 65°C is equivalent to one month of storage at ambient temperatures (25). The extension of the induction period of the oil due to the addition of CE-500, CE-1000 and TBHQ was 2, 2.5 and 2.7 times that of the control, respectively. Furthermore, samples containing CE-500 and CE-1000 had a delayed induction period, comparable to that of BHA/BHT/MGC, which is commonly used in canola oil in Canada. A gradual increase was noticed in the percentage weight gain of all oil samples toward a maximum value with a subsequent decrease during extended storage period. The increase in the weight gain is the addition of oxygen to lipid molecules to form hydroperoxides during primary stages of oxidation. The decrease in weight in later stages may be due to volatilization of some breakdown products of lipid hydroperoxides. Farmer *et al.* (26) and Privett and Nickell (27) have reported that addition of oxygen to lipid to form

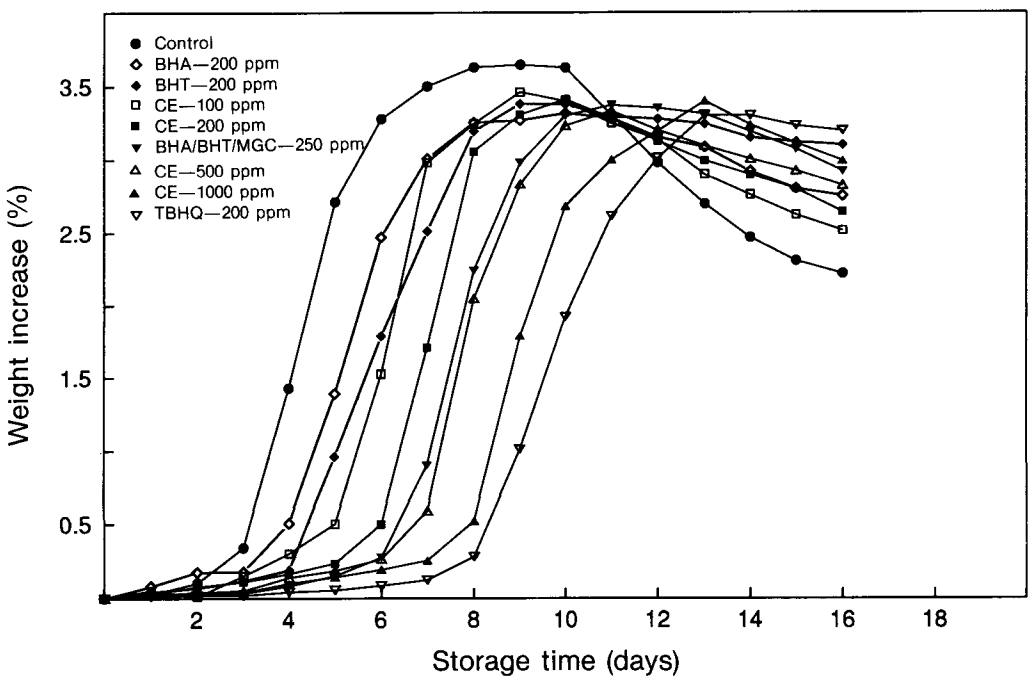

FIG. 1. Effect of canola extract (CE) and synthetic antioxidants on the weight gain of refined-bleached canola oil stored at 65°C. Synthetic antioxidants used were butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), BHA/BHT/monoglyceride citrate (MGC) and *tert*- butylhydroquinone (TBHQ). Weight increases variations are within ±0.01%.

peroxides is reasonably quantitative during the initial stages of autoxidation. The primary purpose of using antioxidants in lipids is to delay a significant accumulation of primary oxidative products, and thus to improve oxidative stability. Addition of CE significantly delayed the accumulation of oxidative products, as shown by low rate of weight gain as compared to other synthetic antioxidants, except TBHQ.

Effect of CE on PV and conjugated diene (CD) values. The addition of CE at 100–1000 ppm to canola oil significantly ($P < 0.05$) decreased the PV and CD during accelerated oxidation. Samples treated with BHA and BHT showed relatively higher PV and CD as compared with CE-treated oils (Figs. 2 and 3). Both of these indices measure primary products of lipid oxidation. These data indicate that an increase in the level of CE paralleled a

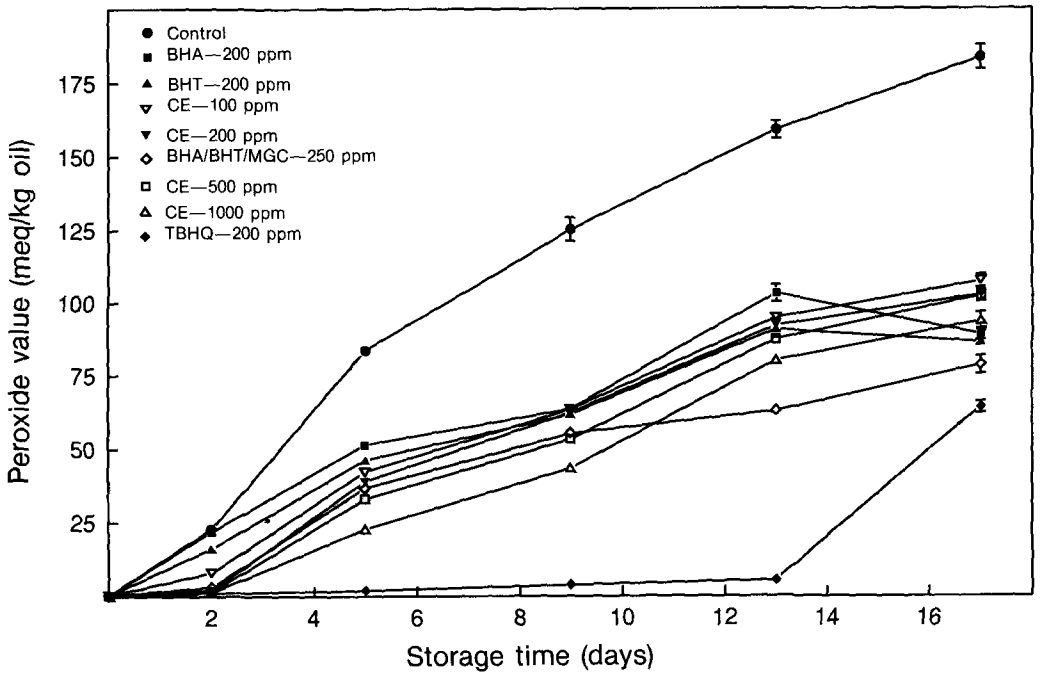

FIG. 2. Effect of CE and synthetic antioxidants on the peroxide value of refined-bleached canola oil stored at 65°C. Refer to Figure 1 for abbreviations.

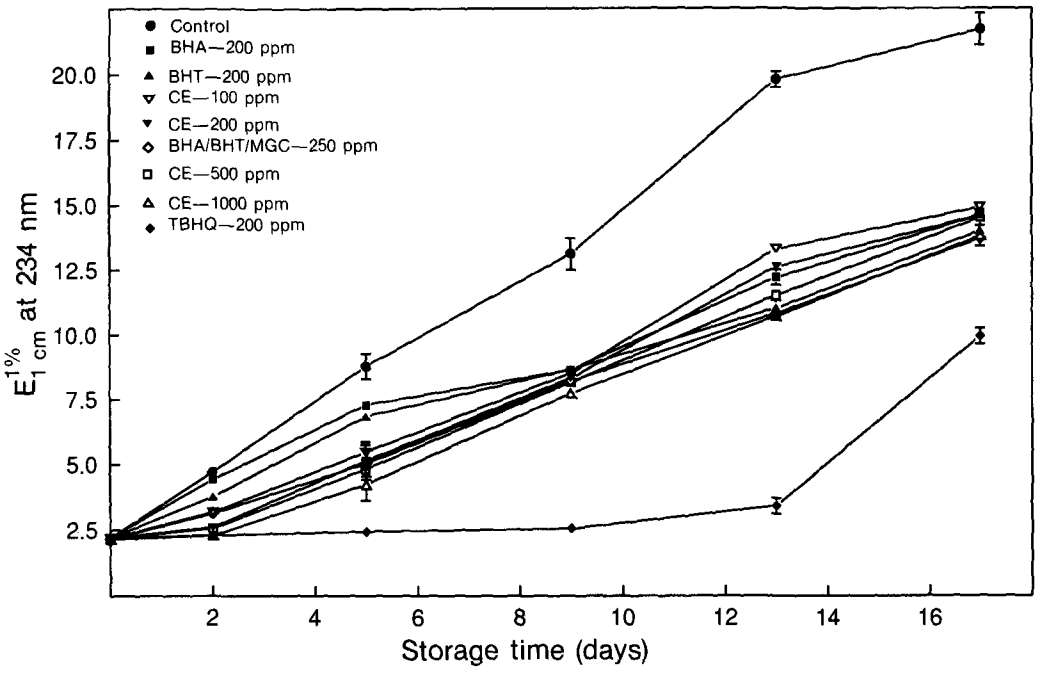

FIG. 3. Effect of CE and synthetic antioxidants on the conjugated diene value of refined-bleached canola oil stored at 65°C. Refer to Figure 1 for abbreviations.

decrease in the formation of both peroxides and conjugated dienes during oxidation. However, PV of canola oil samples treated with 200, 500 and 1000 ppm of CE were similar for up to day 2. After day 5, PV of the samples containing varying concentrations of CE were significantly ($P < 0.05$) different from each other. The CD values of the samples also reflected small differences during the initial stages of oxidation. During later stages, however, TBHQ and CE-1000-treated samples had a significant ($P < 0.05$) effect on lowering CD formation. For up to 17 d, the PV of the control sample increased from 0.37 meq/kg (fresh oil) to 183.4 meq/kg (oxidized oil). Corresponding values for oils treated with CE-500 and CE-1000 were much smaller, changing from 0.30 to 102.1 and from 0.32 to 93.5 meq/kg, respectively. CE was most effective at the 1000 ppm level and gave much lower PV and CD than the control, BHA, BHT, BHA/BHT/MGC and other CE levels. The lowest values for both indicators, however, were observed for TBHQ-treated oils.

Since hydroperoxides are the primary products of lipid oxidation (28), PV provides a clear indication of the oxidative state of vegetable oils. Because of instability of peroxides in the oxidation pathway, however, measurement of PV only provides information about the initial oxidation potential of the oil. Similarly, the CD measures the degree of formation of primary products of lipid oxidation due to the shift in double-bond position upon oxidation of lipid dienes or polyenes (29). A good correlation existed between CD and PV (linear correlation coefficient 0.966 to 0.997). Jackson (30) indicated that formation of hydroperoxides normally coincides with CD formation in oils upon oxidation. Farmer and Sutton (31) indicated that CD values correlate well with hydroperoxide values. St. Angelo *et al.* (32) have suggested that CD values can be used as an index of stability for lipid-containing foods.

Effect of CE on TBARS formation. Among the additives tested, TBHQ was most effective in retarding TBARS formation at a 200-ppm level (Fig. 4; standard deviations in all cases were less than ±0.05 μmol/g oil). Superior activity of TBHQ (200 ppm) to lower TBARS of stored canola oil has been reported in the literature (33). However, this antioxidant is not yet licensed for food use in Canada or in Europe. The effect of the addition of CE (>200 ppm) to RB canola oil was better than that of BHA and BHT and equivalent or slightly better than that of BHA/BHT/MGC. At a 1000-ppm level, CE lowered the content of TBARS more effectively than did BHA/BHT/MGC, even after 17 d of storage. Oil treated with CE-1000 showed a 46, 52, 40, 49 and 56% reduction in TBARS on days 2, 5, 9, 13 and 17, respectively, while BHA/BHT/MGC-treated samples showed a corresponding reduction of 38, 23, 34, 44 and 50% (Fig. 4). TBARS measure the formation of secondary oxidation products, mainly aldehydes (or carbonyls), which may contribute to the off-flavor of oxidized oil. Results of this study indicate that CE has a marked effect in retarding the formation of TBARS of canola oil, better than that of the commonly used BHA/BHT/MGC mixture.

Effect on TOTOX value. The TOTOX values of treated oil samples were lower than that of the control sample (Fig. 5; standard deviations in all cases were less than ±5 units). Oil samples treated with 200 ppm CE had 50% lower TOTOX values up to 13 d of storage as compared with those of the control sample. The lowest TOTOX

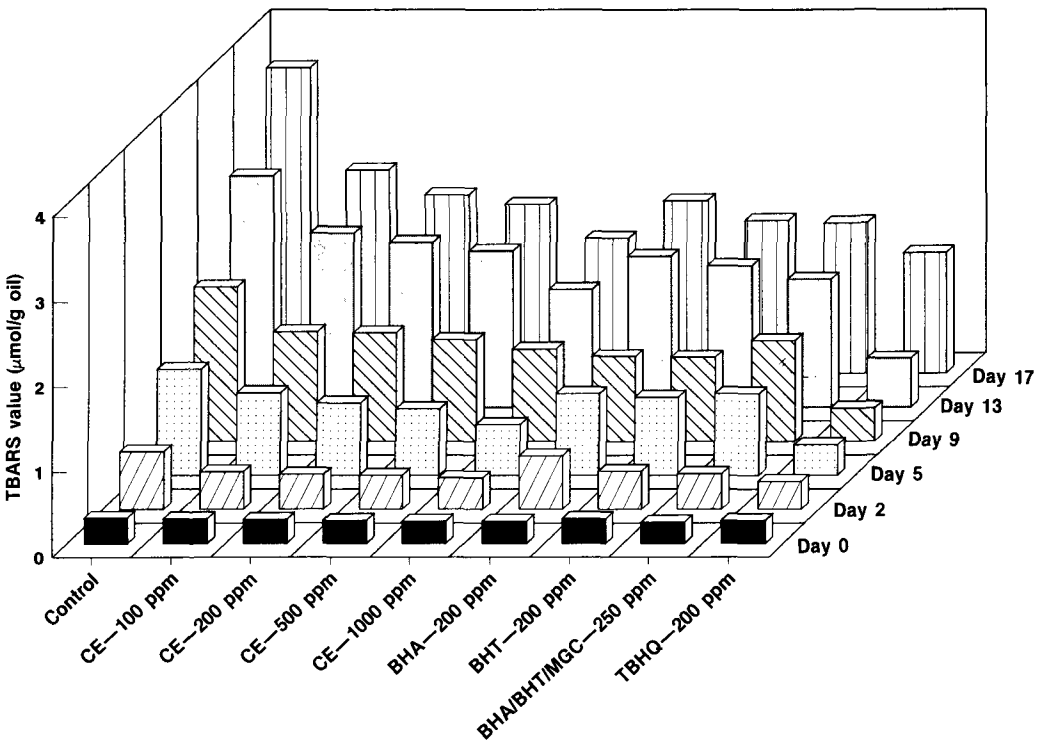

FIG. 4. Effect of CE and synthetic antioxidants on the 2-thiobarbituric acid reactive substances (TBARS) values of refined-bleached canola oil stored at 65°C. Standard deviations in all cases were less than ±0.05 μmol/g oil. Refer to Figure 1 for abbreviations.

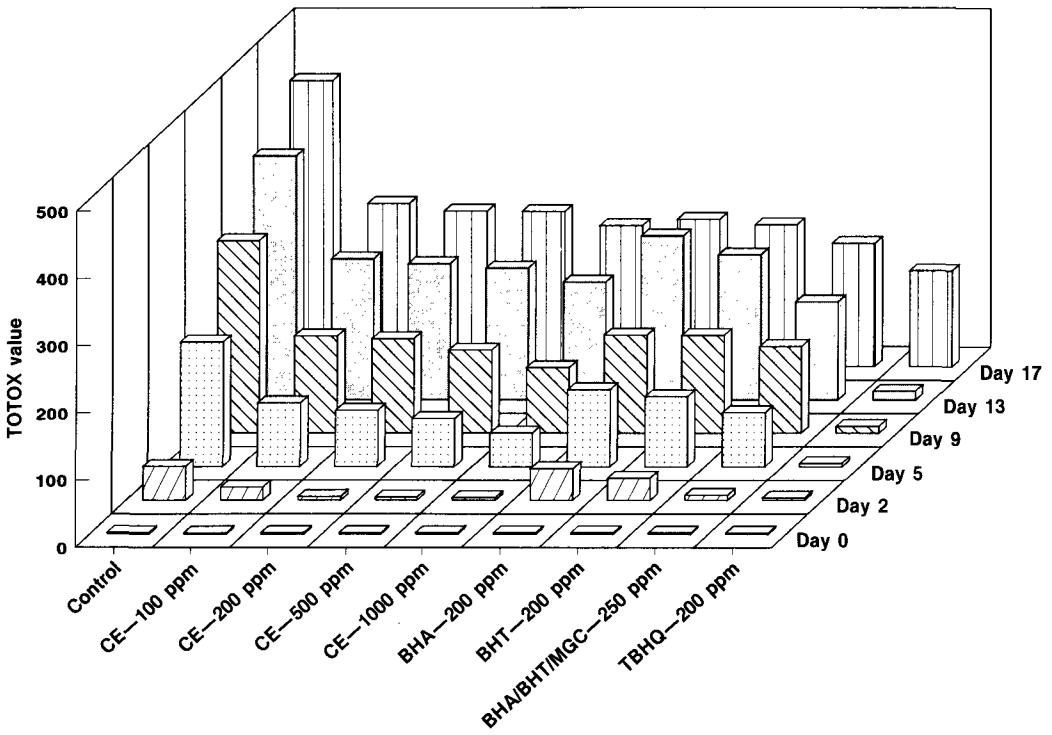

FIG. 5. Effect of CE and synthetic antioxidants on the total oxidation (TOTOX) value of refined-bleached canola oil stored at 65°C. Standard deviation in all cases was less than ±5 units. Refer to Figure 1 for abbreviations.

values were found when 1000 ppm CE was used, resulting in 90, 73, 65, 52 and 50% inhibition on days 2, 5, 9, 13 and 17 of storage under Schaal oven test conditions, respectively. However, as compared with BHA/BHT/MGC after 9 d, CE-1000-treated oil had a slightly higher TOTOX value, likely because of higher PV during the later stages of storage. The effectiveness of CE at 500 and 1000 ppm levels was better than that of BHA (200 ppm), BHT (200 ppm) and BHA/BHT/MGC (250 ppm). Changes in TOTOX values provide practical information regarding progression of formation of primary and secondary oxidation products; however, on a molecular basis, it does not have any meaningful interpretation. Nonetheless, the TOTOX value is often considered by many investigators to be a useful indicator of oxidation of oils because it combines evidence about the past history (*p*-AnV) with the present state (PV) of the samples under investigation (34).

Previous literature is replete with reports of extracts from natural sources that have demonstrated strong antioxidant activity. These extracts have been reported to be more effective in many instances than commonly used synthetic antioxidants (35,36). Ethanolic extracts of navy bean hulls (37), methanolic extracts of oat hulls (38), cottonseed (9) and peanut hulls (39) and aqueous extract of soybean flour (40,41) have shown strong antioxidative activity in vegetable oils, β-carotene-linoleate and meat model systems. Consumers may prefer natural food additives to synthetic compounds because they occur in nature and in foods that have been consumed for thousands of years and are presumed to be safe (42). However, "natural" should not be taken as synonymous with safe. Most natural antioxidative compounds from plant origin are of phenolic nature.

The crude CE possesses good antioxidative properties as evidenced by weight gain, PV, CD, TBARS and TOTOX values of the RB canola oil stored at 65°C. Apart from being a stronger antioxidant, CE did not impart any visible color or perceivable odor to the treated canola oil. These qualities project the potential of an ethanolic extract of canola meal as a natural antioxidant for use in canola oil and, possibly, other lipid-containing foods.

ACKNOWLEDGMENT

We are grateful to the Canola Council of Canada for partial financial support.

REFERENCES

1. Sherwin, E.R., and B.M. Luckadoo, J. *Am. Oil Chem. Soc. 47*:19 (1970).
2. Sheppard, A.J., J.L. Iverson and J.L. Weihruch, in *Handbook of Lipid Research, Fatty Acids and Glyceride*, edited by A. Kuksis, Plenum Press, New York, 1978, pp. 341-379.
3. Tokarska, B., Z.J. Hawrysh and M.T. Clandinin, *Can. Inst. Food Sci. Technol. J. 19*:130 (1986).
4. Ito, N., A. Hagiwara, M. Shibata, T. Ogiso and S. Fukushima, *Gann. 73*:332 (1982).
5. Marshell, W.E., *Food Technol. 28*:50 (1974).
6. Pratt, D.E., C.D. Pietro, W. Porter and J.W. Giffee, *J. Food Sci. 47*:24 (1981).
7. Pratt, D.E., and E.E. Miller, *J. Am. Oil Chem. Soc. 61*:1064 (1984).
8. Whittern, C.C., E.E. Miller and D.E. Pratt, *Ibid. 61*:1075 (1984).
9. Fukuda, Y., T. Osawa, M. Namiki and T. Ozaki, *Agric. Biol. Chem. 49*:301 (1985).

10. Shahidi, F., in *Proceedings of the 14th International Conference of Group Polyphenols*, August 16–18, 1988, St. Catharines, Ontario, pp. 361–362.
11. Shahidi, F., and P.K.J.P.D. Wanasundara, *CRC Crit. Rev. Food Sci. Nutr. 32*:67 (1992).
12. Kozlowska, H., M. Naczk, F. Shahidi and R. Zadernowski, in *Canola and Rapeseed, Production, Chemistry, Nutrition and Processing Technology*, edited by F. Shahidi, Van Nostrand Reinhold, New York, 1990, pp. 193–210.
13. Zadernowski, R., *Acta Acad. Agricult. Techn. Olst. Technol. Alimen. 21*:4 (1987).
14. Zadernowski, R., H. Nowak and H. Kozlowska, in *Rapeseed in Changing World. Proceedings of the 8th International Rapeseed Congress*, Vol. 3, edited by D.I. McGregor, July 9–11, 1991, Saskatoon, pp. 883–887.
15. Shahidi, F., and M. Naczk, *J. Am. Oil. Chem. Soc. 62*:917 (1992).
16. Clandinin, D.R., and A.R. Robblee, *Ibid. 58*:682 (1981).
17. Keough, K.M.W., and N. Kariel, *Biochim. Biophys. Acta. 902*:11 (1987).
18. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1990.
19. Olcott, H.S., and E. Einset, *J. Am. Oil Chem. Soc. 35*:161 (1958).
20. *Standard Methods for the Analysis of Oils and Fats and Derivatives*, 7th edn., edited by C. Paquot, and A. Hautfenne, Blackwell Scientific Publishers Ltd., Oxford, 1987.
21. Wanasundara, U.N., and F. Shahidi, *J. Food Lipids 1*:15 (1993).
22. Snedecor, G.W., and N.G. Cochran, *Statistical Methods*, 7th edn., The Iowa University Press, Ames, 1980, pp. 365–371.
23. *SAS User's Guide: Statistics*, SAS Institute Inc., Cary, 1990.
24. Vaisey-Genser, M., and N.A.M., Eskin, *Canola Oil—Properties and Performance*, Canola Council of Canada, Winnipeg, Manitoba, 1982, pp. 8–11.
25. Evans, C.D., G.R. List, H.A. Moser and J.C. Cowan, *J. Am. Oil Chem. Soc. 50*:218 (1973).
26. Farmer, E.H., H.P. Koch and D.A. Sutton, *J. Chem. Soc.*:119 (1943).
27. Privett, O.S., and C. Nickell, *J. Am. Oil Chem. Soc. 33*:156 (1956).
28. Labuza, T.P., *CRC Crit. Rev. Food Technol. 2*:355 (1971).
29. Logani, M.K., and R.E. Davis, *Lipids 15*:485 (1980).
30. Jackson, H.W., *J. Am. Oil Chem. Soc. 58*:227 (1981).
31. Farmer, E.H., and D.A. Sutton, *Trans Faraday Soc. 42*:228 (1946).
32. St. Angelo, A.J., R.L. Ory and L.E. Brown, *J. Am. Oil Chem. Soc. 52*:34 (1975).
33. Hawrysh, Z.J., L.M. McMullen, C. Lin, B. Tokarska and R.T. Hardin, *Can. Inst. Food Sci. Technol. J. 23*:94 (1990).
34. Rossell, J.B., in *Rancidity in Foods*, edited by J.C. Allen, and J. Hamilton, Applied Science Publishers, London, 1983, pp. 21–45.
35. Osawa, T., and M. Namiki, *Agric. Biol. Chem. 45*:735 (1981).
36. Ramarathnam, N., T. Osawa, M. Namiki and S. Kawakishi, *J. Agric. Food Chem. 36*:732 (1988).
37. Onyenebo, S.N., and N. Hettiarachchi, *Ibid. 39*:1701 (1991).
38. Duve, K., and P.J. White, *J. Am. Oil Chem. Soc. 68*:365 (1991).
39. Duh, P., D. Yeh and G. Yen, *Ibid. 69*:814 (1992).
40. Rhee, K.S., Y.A. Ziprin and K.C. Rhee, *J. Food Sci. 44*:1132 (1979).
41. Ziprin, Y.A., K.S. Rhee, Z.L. Carpenter, R.L. Hostetlet, R.N. Terrel and K.C. Rhee, *Ibid. 46*:58 (1981).
42. Dugan, L.R., in *Autoxidation in Food and Biological Systems*, edited by M.G. Simic, and M. Karel, Plenum Press, New York, 1980, pp. 261–282.

[Received September 2, 1993; accepted March 14, 1994]