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Effect of Navy Bean Hull Extract on the Oxidative Stability of Soy and Sunflower Oils

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The effectiveness of freeze-dried navy bean hull extract (NBHE) as an antioxidant was evaluated in storage studies with soy and sunflower oils. Monthly peroxide value (PV) determinations indicated NBHE to be a stronger antioxidant than butylated hydroxyanisole–butylated hydroxytoluene (BHA–BHT) mixture and rosemary AR but was less effective than *tert*-butylhydroquinone (TBHQ) at all levels (100, 500, and 1000 ppm) and storage conditions (26 and 37 °C). At the 100 ppm level, NBHE-treated soy oil attained a PV of 25.4 mequiv/kg at 37 °C in 9 months, while the control oil sample had a PV of 62.0 mequiv/kg at the same period. At 26 °C, the same concentration of NBHE lowered the PV of soy oil to 14.8 mequiv/kg compared to a PV of 32.7 mequiv/kg attained by the control oil sample for 12 months under the same condition. Similar pattern of PVs were observed in sunflower oil treated with NBHE and stored under the same temperature conditions.

INTRODUCTION

In vivo, fats and oils are known to be protected by several antioxidants and biochemical pathways that ensure their chemical and biochemical integrity. However, when a plant is harvested or an animal is slaughtered for food purposes, fat and oil contents of living tissue begin to deteriorate. This deterioration is of utmost importance in processed foods containing fats and oils, where they lead to rancidity. Among the important causes of rancidity are oxidation reactions, microbial activities, and enzyme-catalyzed hydrolysis of fats (Robards et al., 1988).

Antioxidants form a major means of preserving our food supply (edible fats, oils, and fat-containing foods) from the development of objectionable flavors and odors. These are caused by oxidative deterioration of fats that leads to the formation of decomposition products which may form toxic polymers (Johnson, 1971; Sims and Fioriti, 1980). Malonaldehyde, which is one of the products of lipid oxidation, has been shown to be carcinogenic. Shermberger et al. (1974) reported that mice initiated with malonaldehyde and then promoted with cotton oil had about an 85% incidence of tumor, while about 50% of the animals tested with malonaldehyde as an initiator had liver or rectal tumors. The literature is replete with reports of plant extracts that have demonstrated strong antioxidant activity. These papers indicate, in many instances, that the extracts were more effective than some major synthetic antioxidants (Sherwin, 1990; Dugan, 1980; Pratt, 1980; Dewdney and Meara, 1977). Shulze et al. (1971) investigated the antioxidant effects of extracts from spices. Extracts from rosemary, nutmeg, and sage were tested for antioxidant activities; rosemary extract proved to be the most potent. Chang et al. (1977) found purified extracts of rosemary to be effective inhibitors of reversion in soybean oil and to inhibit formation of peroxides in stored potato chips. Nestle S.A. (1983) patented a process for extracting antioxidants from rosemary, sage, and parsley. Rosemary extract has since been commercialized after being approved by the FDA for direct food application.

The antioxidant activity of navy bean hull extract has been demonstrated (Onyeneho, 1990). The objective of this investigation was to examine the effect of navy bean hull extract (NBHE) on the oxidative stability of soy and sunflower oils at both ambient temperature (26 °C) and 37 °C storage conditions.

MATERIALS AND METHODS

Materials. Upland variety of navy beans (*Phaseolus vulgaris*) was purchased in bulk from AgriSales Inc., Casselton, ND. Pure expeller-pressed soy and sunflower oils were purchased from

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Hain Pure Foods Co. Inc., Los Angeles, CA, and Cargill Inc., Des Moines, IA, respectively. Synthetic antioxidants *tert*-butylhydroquinone (TBHQ) and butylated hydroxyanisole–butylated hydroxytoluene (BHA–BHT) mixture (20% BHA and 20% BHT in 60% corn oil) were obtained from Eastman Kodak Co., Kingsport, TN. Rosemary AR, a natural antioxidant extract, was obtained from Culinar Corp., Fjalkinge, Sweden. All other chemicals were common laboratory reagents of analytical grade.

Methods. *Antioxidant Extract Preparation.* Navy beans (*P. vulgaris*) were cleaned by hand-picking foreign materials, washed, and conditioned for 72 h at 38 °C in a convection-type seed dryer. This lowered the moisture content from 13.5% to 10%. The conditioned beans were passed through the first break rolls of the Allis-Chalmers mill at a roll setting of 3.5, which primarily cracked the beans open to release the hulls. The mill stock was sequentially passed through U.S. 18, 46, and 60 sieves to separate and to remove the maximum amount of endosperm material from the hulls. The hulls obtained were aspirated and ground in a Wiley mill to pass through a 0.5-mm screen. Ground hulls were defatted three times with 4 volumes of petroleum ether by shaking for 2 h in an Eberbach shaker and filtered. The residue was dried under a hood until all traces of petroleum ether were removed. The defatted, ground hulls were shaken for 2 h with 5 volumes of 95% ethanol and filtered through Whatman No. 4 paper. The extraction was repeated twice, and the combined filtrates were subjected to vacuum distillation at 40 °C to remove the ethanol. The aqueous extract was autoclaved at 115 °C and 15 psi for 15 min and then freeze-dried (−60 °C, 60-μm vacuum) for 96 h to obtain the antioxidant extract.

Preparation of Oils for Storage. Three levels of the freeze-dried NBHE, 100, 500, and 1000 ppm, were used to test its antioxidant effectiveness. The freeze-dried extract (200 mg) was homogeneously mixed with 25.0 g of a 2.0-kg portion of pure expeller-pressed soy oil in an ice bath by using a BraunSonic Model 2000 sonicator. The resulting emulsion was added to the remainder of the 2.0 kg of oil and mixed for 15 min. Soy oils containing 500 and 1000 ppm were similarly prepared. Thirty-gram portions of oil each containing 100, 500, and 1000 ppm of NBHE were aliquoted into 25-mL screw-cap tubes to the brim and capped after the tubes were sealed with parafilm to exclude any headspace. Twenty-four tubes containing oil of each level of NBHE were packaged in cardboard boxes and stored at ambient temperature (26 °C) for 12 months. Thirty-six similarly packaged tubes containing oil of each NBHE level were stored at 37 °C for 9 months. Peroxide value determinations in duplicate were conducted at zero time (before storage) and at monthly intervals. This additive remained dispersed in oil through the study period. Soy oils containing 100, 500, and 1000 ppm each of BHA–BHT mixture, TBHQ, Rosemary AR and control samples (oil without additives) were similarly prepared, packaged, and stored at the two temperature conditions. Duplicate samples were analyzed for PVs at zero time and at monthly intervals.

This study was repeated by substituting soy oil with pure expeller-pressed sunflower oil.

Chemical Analysis. *Peroxide Value.* The peroxide value (PV) (expressed as milliequivalents of peroxide per kilogram of fat) was determined in duplicate by the iodometric titration procedure (AOCS Method Cd 8-53). A sample size of 5.0 g was used.

Statistical Analysis. The peroxide values were analyzed by the General Linear Models (GLM) program for analysis of variance and regression estimation (SAS Institute, 1985).

RESULTS AND DISCUSSION

When soy oil was treated with freeze-dried navy bean hull extract (NBHE) and stored at either ambient temperature (26 °C) for 12 months or 37 °C for 9 months, lower peroxide values (PVs) were observed than without it (Table I). Similar lower PVs were observed when sunflower oil was treated with NBHE and stored under the same conditions (Table II). These data demonstrated that NBHE was acting as an antioxidant in these vegetable oils. No significant differences were observed among the three levels (100, 500, and 1000 ppm) used in the investigation ($p > 0.05$).

Table I. Effect of Varying Levels of Navy Bean Hull Extract (NBHE) and Other Antioxidants on the Peroxide Value (PV) of Soy Oil Stored for 12 Months at Ambient Temperature (26 °C) and for 9 Months at 37 °C

	PV,[a] mequiv/kg	
additive/concn	26 °C	37 °C
NBHE-1[b]	14.8 ± 2	25.4 ± 2
NBHE-2	14.3 ± 1	20.0 ± 0.5
NBHE-3	14.0 ± 1	20.0 ± 0.5
BHA-BHT-1	17.2 ± 3	32.0 ± 3
BHA-BHT-2	17.0 ± 2	24.6 ± 1
BHA-BHT-3	15.8 ± 1	23.9 ± 1
rosemary-1	21.2 ± 2	26.0 ± 2
rosemary-2	20.0 ± 2	23.6 ± 1
rosemary-3	20.0 ± 2	22.0 ± 1
TBHQ-1	12.5 ± 2	18.0 ● 1
TBHQ-2	12.0 ● 1	17.5 ± 1
TBHQ-3	12.0 ± 1	16.5 ± 1
control	32.7 ± 3	62.0 ± 2
zero time	6.5 ● 0.5	6.5 ± 0.5

[a] Values are means of duplicate analyses. [b] 1, 2, and 3, 100, 500, and 1000 ppm, respectively.

Table II. Effect of Varying Levels of Navy Bean Hull Extract (NBHE) and Other Antioxidants on the Peroxide Value (PV) of Sunflower Oil Stored for 12 Months at Ambient Temperature (26 °C) and for 9 Months at 37 °C

	PV,[a] mequiv/kg	
additive/concn	26 °C	37 °C
NBHE-1[b]	15.9 ● 1	18.6 ● 2
NBHE-2	14.0 ● 1	16.3 ± 1
NBHE-3	13.4 ± 1	14.6 ● 1
BHA-BHT-1	17.5 ± 2	33.0 ± 2
BHA-BHT-2	15.8 ± 1	27.4 ● 3
BHA-BHT-3	15.8 ● 1	20.3 ● 3
rosemary-1	19.3 ± 1	21.9 ● 2
rosemary-2	15.6 ± 1	17.5 ± 2
rosemary-3	15.1 ± 1	16.0 ± 1
TBHQ-1	11.3 ± 2	13.0 ± 1
TBHQ-2	10.8 ± 1	10.6 ● 0.5
TBHQ-3	8.5 ± 1	9.0 ● 0.5
control (no additive)	27.8 ● 1	42.5 ± 3
zero time	1.9 ± 1	1.9 ± 1

[a] Values are means of duplicate analyses. [b] 1, 2, and 3, 100, 500, and 1000 ppm, respectively.

Plots of the relative increases of PV as a function of storage time (months) when soy oil treated with 100 ppm each of NBHE, BHA–BHT mixture, TBHQ, or Rosemary AR and the control stored at 26 and 37 °C are shown in Figures 1 and 2, respectively. Similar plots for sunflower oil are illustrated in Figures 3 and 4. All four plots show a similar pattern of increases in PV with the progression of storage time irrespective of storage conditions. However, greater increases in peroxide formation were observed in oils stored at 37 °C (Figures 2 and 4) than in those stored at 26 °C (Figures 1 and 3). This observation is in agreement with the findings of Sherwin (1978) that heat greatly accelerates oxidation, and it has been estimated that for about a 15 °C increase in temperature the rate of oxidation reaction doubles (Swern, 1964).

These results indicated that NBHE possesses good antioxidant property as evidenced by its greater ability ($p < 0.05$) to decrease the PV of these vegetable oils than the synergistic effect of BHA and BHT and Rosemary AR. However, it was less effective in inhibiting oxidative rancidity development in these oils than TBHQ (Figures 1–4).

The first product of fatty acid or lipid oxidation is a peroxide or hydroperoxide, and it is a very sensitive

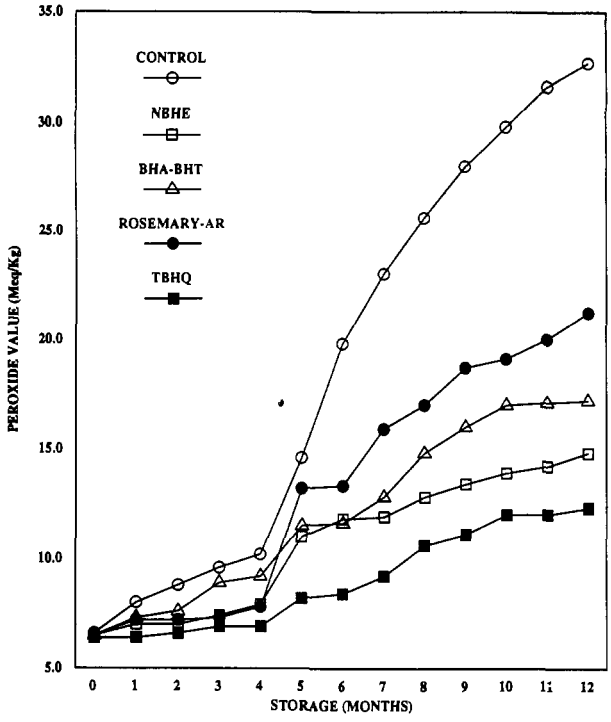

Figure 1. Effect of navy bean hull extract and other antioxidants on the peroxide value of soy oil stored at ambient temperature (26 °C).

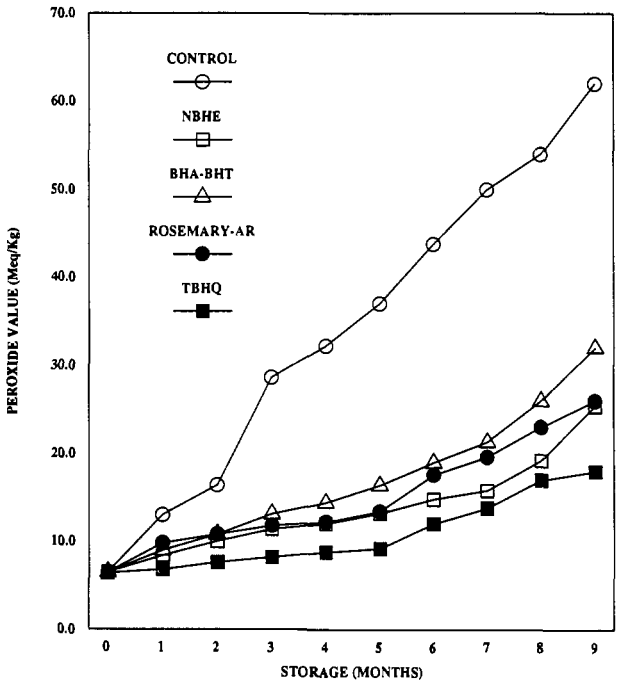

Figure 2. Effect of navy bean hull extract and other antioxidants on the peroxide value of soy oil stored at 37 °C.

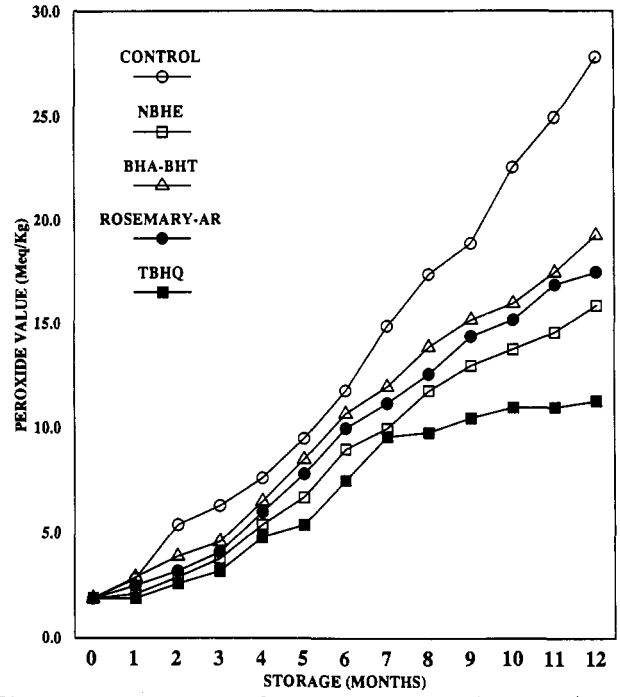

Figure 3. Effect of navy bean hull extract and other antioxidants on the peroxide value of sunflower oil stored at ambient temperature (26 °C).

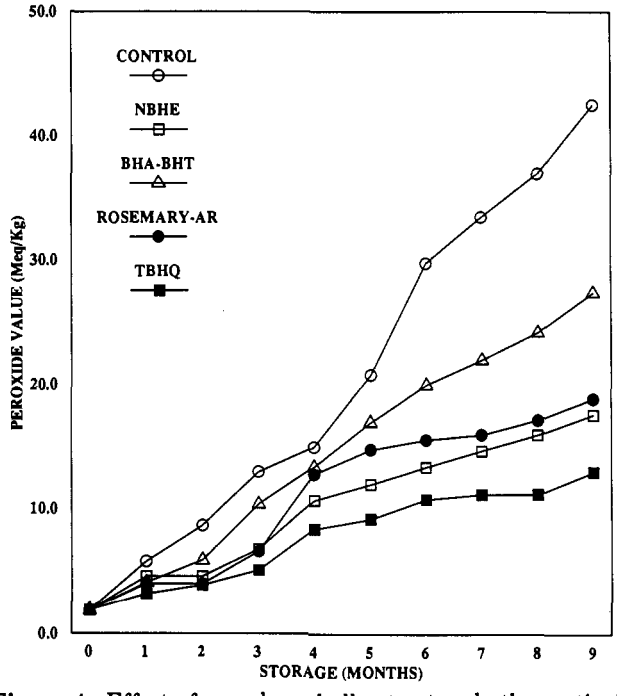

Figure 4. Effect of navy bean hull extract and other antioxidants on the peroxide value of sunflower oil stored at 37 °C.

indicator of the early stages of oxidative deterioration of a fat or oil (Rossel, 1989). Dugan (1955) noted that a PV of 20 for lard or 100 for vegetable oils indicates that they are rancid by organoleptic evaluation. The above data are in agreement with those of Gunstone and Norris (1983). Extracts from natural sources possessing considerable antioxidant property have been reported by various investigators. Consumers prefer them to synthetic antioxidants since they occur in nature and in foods which have been used for thousands of years and are, therefore, presumed to be safe (Dugan, 1980). According to Ikins (1991), food scientists and consumers share a great deal of interest in naturally occurring antioxidants which include aqueous extracts from vegetables, hydrolyzed proteins, amino acids, spices, herbs, and Maillard browning pigments. It is of additional interest to note that apart from its strong antioxidant property NBHE did not impart any visible color or perceivable odor to the oils at the levels used. It is lightly colored and bland to taste. It also dissolves instantly in oil upon sonication to form an emulsion which remained completely dispersed in the oils throughout the storage period. These qualities project NBHE as a potential natural antioxidant for use in vegetable oils or products containing them.

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