

# Antioxidant Activity of Oat Extract in Soybean and Cottonseed Oils

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A previously published method for extracting antioxidants from Noble oats with methanol was modified to improve the antioxidant activity. The extract was tested in soybean and cottonseed oils held at 30 and 60°C in the dark and at 30°C in the light. During storage, the peroxide values (PV) of the oils were generally significantly lower ( $P \leq 0.05$ ) with the addition of the extract than was the control (no additives), and the PV were slightly higher than for oils containing TBHQ. In addition, the extract was added to emulsions of the same oils and held at 30°C in the light and at 60°C in the dark. The PV of the emulsions containing the extract were significantly lower ( $P \leq 0.05$ ) than were the PV of those containing tertiary butylhydroquinone and the control.

**KEY WORDS:** Antioxidant, autoxidation, cottonseed oil, oat, oxidation, soybean oil.

The addition of antioxidants to fats and oils or to foods that contain fats and oils is one of the most efficient ways to prevent oxidation of the lipids. There is a concern about the possible toxicity of synthetic antioxidants (1), so the popularity of natural antioxidants has increased. Although there is no assurance of the safety of natural antioxidants, there is some comfort knowing that such antioxidants were purified from natural products that have been consumed for generations.

The utilization of natural antioxidants from oat was first reported by Musher (2–5). The ground or aqueous extracts of cereals, including oat, and oilseeds effectively prevented lipid oxidation at both room and accelerated room temperatures (2–5). Musher (4) claimed that oat flour increased the stability of oils, fats, margarine and mayonnaise. The oat flour also was effective when dusted over bacon and potato chips.

Daniels and Martin (6) isolated and purified ferulic and caffeic acids from oat. The antioxidant activity of an oat extract containing these phenolics was as effective as propyl gallate (PG) and butylated hydroxytoluene (BHT) as measured by a recording oxygen apparatus (6). Further work showed that the extract could be separated into 24 active fractions by thin-layer chromatography (TLC) and column chromatography (CC). Some of the fractions were identified as caffeic and ferulic acid esters and monoesters of C<sub>26</sub> and C<sub>28</sub>,  $\alpha$ ,  $\omega$ -diols (6), and monoesters of hexacosan-1-ol, 26-hydroxyhexacosanoic acid and 28-hydroxyoctacosanoic acid (7–9). Glycerol monoesters of 26-hydroxyhexacosanoic acid and 28-hydroxyoctacosanoic acid also were found (8,9). Collins *et al.* (10,11) found a group of cinnamic acid conjugates, namely avenanthramides. The structure of 10 components in this group were elucidated by TLC and CC, mass spectrometry (MS), nuclear magnetic resonance (NMR) and ultraviolet absorption spectroscopy (UV).

Solvent extraction is the major method used to isolate natural antioxidants. Supova *et al.* (12) reported that various solvent extracts of oat had antioxidant activity in lard when tested by the active oxygen method. Also, solvents with higher polarity yielded greater antioxidant activity, and a

methanolic extract of defatted oat flour was the most active. Chang *et al.* (13), in a patent for extraction of antioxidants from rosemary and sage, found methanol and ethanol to be the most successful solvents. Duve and White (14) compared the activity of eight solvent extractions of oats and concluded that the greatest antioxidant activity was derived from the methanol extracts of undefatted oat.

The objectives of the current study were threefold. The first was to determine an improved method to extract and concentrate the antioxidants from oat. The second was to verify the presence of phenolic antioxidants as the active components in the extract by TLC, gas chromatography (GC) and by GC/MS. The third objective was to test the antioxidant activity of the extract in soybean and cottonseed oils and their emulsions under different storage conditions.

## EXPERIMENTAL PROCEDURES

*Extraction of oat antioxidants.* Noble oats (*Avena sativa* L.) were grown near Ames, Iowa, in 1991 and 1992. After harvest, the dehulled oat groats were stored at 4°C and 45% relative humidity until needed for extraction. The groats were ground, and the resulting powders were passed through a No. 40 U.S. standard mesh screen.

The extraction of antioxidants and  $\Delta^5$ -avenasterol from oat was done by the method of Duve and White (14) with some modifications. As previously described (14), oat flour (1 kg) was extracted with methanol (4 L) at room temperature with constant stirring for 24 h. The solvent was changed every day for seven days. At every solvent change, the mixture was filtered through Whatman #4 filter paper. The filtrates were combined and evaporated in a rotary evaporator to 30 mL at 45°C.

Modification of the method of Duve and White (14) involved fractionating the crude extract through a silicic acid column (15 mm  $\times$  220 mm). The silicic acid (100 mesh; Aldrich Chemical Company, Milwaukee, WI) was activated overnight at 120°C, then washed first with 400 mL methanol three times and then with 400 mL hexane three times. A hexane slurry of the treated silicic acid (200 g) was then packed into the column, and 15 mL of methanolic extract was applied to the top. The column was eluted stepwise with 500 mL hexane and 500 mL methanol. The two separated fractions were then rotary-evaporated to 30 mL each at 45°C and stored under nitrogen at -10°C until analyzed.

*Determination of total phenolic contents (TPC).* TPC of the extract was tested by Method (9.110) of the Association of Official Analytical Chemists (AOAC) (15). Briefly, oat extract (0.1 mL) was added into a 100-mL volumetric flask containing 75 mL distilled water. Folin-Denis reagent (5 mL) and saturated sodium carbonate solution (10 mL) were added to the flask and diluted to 100 mL with distilled water. The mixture was then shaken for 1 min and allowed to stand at room temperature for 30 min. The absorbance of the solution was viewed in a spectrophotometer at 760 nm.

*Oil storage tests.* All storage tests were conducted with refined, bleached and deodorized soybean and cottonseed

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oils obtained from commercial sources. The oils contained no additives except citric acid. All tests were run on duplicate oil samples. Oat extract (0.005, 0.02 and 0.03%, wt/wt based on TPC of the extract placed in the oil) was partly dried under nitrogen first and then added to 10 g of cottonseed oil stored at 30°C for 30 d or at 60°C for 25 d in the dark. Oat extract at slightly different concentrations than just listed (0.01, 0.02 and 0.03%) was added to the cottonseed and soybean oils stored at 30°C under fluorescent light at a distance of 180 foot-candles (ft-c) for 10 d and to soybean oil stored at 60°C in the dark for 10 d. Additional treatments for each test included tertiary butyl hydroquinone (TBHQ) (0.02%), the best synthetic antioxidant available (16), and a control containing no additives (except citric acid). All treatments were stored in open beakers (50 mL) that had been pre-cleaned with a potassium ethanol solution (5 g potassium hydroxide per 100 mL ethanol). The oils were sampled every two days.

**Emulsion storage tests.** The antioxidant activity of the oat extract also was tested in soybean and cottonseed oil emulsions. All tests were run on duplicate emulsion preparations. The emulsions consisted of 10 g (56%) oil blended with 7.7 g (43%) water and 1 g (1%) Tween 20, an emulsifier, (Aldrich). Oat extract (0.01, 0.02 or 0.03%) or TBHQ (0.02%) was added to each treatment mixture and blended in a Waring commercial blender (Dynamics Corporation of America, New Hartford, CT) for 1 min to form stable emulsions. A control treatment with no additives also was tested. The emulsions were then stored at 30°C under fluorescent light at a distance of 180 ft-c or at 60°C in the dark for up to 10 d. All treatments were stored in open beakers (50 mL) that had been pretreated with potassium ethanol solution as previously described. The emulsions were sampled every two days.

**Peroxide values (PV).** The PV of the oils were analyzed on the day of sampling by the Stamm test as modified by Hamm *et al.* (17). The PV of the emulsions were analyzed according to AOCS Standard Method cd 8-53 (18). Because the emulsions contained water, the Stamm test was not suitable for PV measurements. The PV of the oils and emulsions were run in triplicate and duplicate, respectively.

**GC of fatty acid methyl esters (FAME).** Soybean and cottonseed oils and emulsions from the storage tests were analyzed for fatty acid composition on a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Kennett Square, PA) equipped with a flame-ionization detector and split/splitless injector. A DB-23 fused silica capillary column was used with dimensions of 0.25 mm × 15 m × 0.25 μm film thickness (J&W Scientific Inc., Rancho Cordova, CA). Chromatographic parameters were set as follows: injector temperature, 250°C; detector temperature, 250°C; column temperature programming, 140 to 200°C at 12°C/min with 5°C min holding time at 200°C; and carrier gas (He) at 100 mL/min. The fatty acids were converted to FAME by following the procedure of Hammond and Fehr (19). All tests were run in duplicate, and the results were averaged.

**TLC for antioxidant activity.** The method of Pratt and Miller (20) was used to estimate the antioxidant activity of the oat extracts. The TLC plates (0.25 mm) precoated with silica gel G (Fisher Scientific, Itasca, IL) were activated at 120°C for 2 h. Oat extract (25 μL) was streaked

on the plates and developed in the upper phase of chloroform/ethanol/acetic acid (98:2:2). After development, the plates were dried and sprayed with a β-carotene solution. The β-carotene (9 g) was dissolved in 30 mL chloroform and mixed with two drops of linoleic acid and 60 mL ethanol. The intensity of orange color corresponded to the antioxidant activity of the oat extract (20). During the preliminary stages, the ground groats, whole groats and hulls were extracted with methanol. The methanolic extract of ground groats had the best antioxidant activity; therefore, it was chosen for further study.

**TLC to identify the chemical composition of the oat extract.** The chemical composition of the oat extract was tentatively determined by following a modified procedure of Taga *et al.* (21). The purified extract (50 μL) obtained after CC, was streaked on a TLC plate (0.25 mm) and developed first in the solvent system of chloroform/ethanol/acetic acid. The plate was viewed under UV light (360 nm) and then sprayed with β-carotene along one side of the plate to identify bands of antioxidants. The clean portions of the separated bands (positive in β-carotene spray) were scraped from the plate, extracted in methanol and concentrated to 1.5 mL. The extracts of each band (10 μL) were spotted on another TLC plate and developed in *n*-butanol/acetic acid/water (4:1:5). Nine sprays were used to help identify the composition of the bands (Table 1) (22–30).

**GC and GC/MS to identify the chemical composition of the oat extract.** The oat extract was analyzed for its phenolic acid composition. The extract was first hydrolyzed and then derivatized with trimethylsilyl (TMS) by the method of Pometto and Crawford (31). The gas chromatograph was identical to that described for analysis of FAME. The MS was a Hewlett-Packard 5970 mass-selective detector. A SPB-1 fused-silica capillary column was used with dimensions of 0.25 mm × 25 m × 0.25 μm film thickness (Supelco Inc., Supelco Park, Bellefonte, PA). Chromatographic parameters were set as follows: injector temperature, 240°C; detector temperature, 260°C; column temperature programming, 120°C to 260°C at 12°C/min with 2 min holding at 140°C and 10 min holding at 260°C; and column flow, 1.74 mL/min.

The following standards (Sigma Chemical Company, St. Louis, MO) were converted to TMS derivatives and then analyzed by GC and GC/MS (31): ferulic acid, caffeic acid, *trans*-cinnamic acid, *o*-coumaric acid, 3,5-dimethoxy-4-hydroxy coumaric acid, protocatechuic acid, syringic acid, syringaldehyde, genistic acid, vanillin, *p*-hydrobenzoic acid, vanillic acid and 3,4-diethoxybenzoic acid. The GC/MS and retention time of unknown compounds in the extract were compared with those of the standards to verify identification.

**Data and statistical analyses.** All data are the average of two replicate experiments. The least-square means of the PV and individual fatty acid contents were calculated by the Statistical Analysis System (SAS) (32). The significance was accepted at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

**Preliminary storage tests with unmodified oat extract.** Methanolic oat extracts, prepared exactly as described by Duve and White (14), were added to soybean oil and stored. As before, there were no apparent differences in

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TABLE 1

Sprays Used in Antioxidant Identification by Thin-Layer Chromatography and Results for Bands Testing Positive

Sprays	Band	Color	Identified components (reference number)
FeCl <sub>3</sub> -K <sub>3</sub> Fe(CN) <sub>6</sub>	A,B,C	Blue	Phenolics (22)
FeCl <sub>3</sub>	A,B,C	Red/brown	Phenolics without <i>o</i> / <i>p</i> -OH <sup>a</sup> (23)
NH <sub>4</sub> OH-AgNO <sub>3</sub>	B,C	Brown/black/gray	Reducing compounds (24)
Van-pts	A	Red-violet	Flavonoids phloroglucinol nucleus
	B,C	Pink	Flavonoids resorcinol nucleus (25)
DPNA <sup>b</sup>	A,B,C	Brown	Free <i>o</i> / <i>p</i> -OH phenolics <sup>a</sup> (26)
Na <sub>2</sub> CO <sub>3</sub>	B,C	Fluorescent color changes	Free OH phenolics (27)
I <sub>2</sub> vapor	A,B,C	Brown	Sugar mercaptals, alcohols, hexanoic acids, glycerides, <i>N</i> -acylamino sugars, neutral and acid polysaccharides (28)
Aniline oxalate	A	Green-brown	Hexoses
	B	Red	Pentoses
	C	Yellow	Uronic acids (29)
<i>p</i> -Anisidine-HCl	A,B	Light brown	Deoxysugars, aldohexoses
	C	Brown	Aldopentoses (30)

<sup>a</sup>Phenolics with free *ortho*- or *para*-hydroxy groups.<sup>b</sup>Diazotized *p*-nitroaniline.

effectiveness among the treatments, containing extracts and the control with no additives, when stored at 60 °C for up to 20 d. Data are not shown. Modifications of the extract as described in the Materials and Methods section revealed more promising antioxidative potential as measured by TLC, so the extract was further tested as described in this paper.

**TPC tests.** The TPC of each change of methanol from the oats was determined (Table 2). The first solvent change resulted in the greatest amount of phenolic material. Generally, the remaining phenolic content was reduced to half in each subsequent extraction. From the fifth to the seventh solvent change, the phenolic contents were very low. The TPC of the hexane eluant from CC of the crude extract also was tested, and only a small amount of phenolics was found. These results again confirmed that nonpolar solvents were not effective in extracting phenolics. The TPC of the methanolic eluant decreased slightly, from 30.5 ppm before CC to 30.1 ppm after CC.

About 50% of the extract consisted of dry material, which included the phenolics and, likely, nitrogenous impurities (7). Preliminary tests revealed a decrease in anti-

oxidant activity if the extract became dry. For example, the methanolic eluant from CC was evaporated to dryness and tested for TPC. A reduction in TPC of the extract occurred from 30 ppm before drying to 11 ppm after drying, likely because of the oxidative decomposition of phenolics when dried and exposed to air. Therefore, the extract always was stored in solvent and under nitrogen.

**TLC for antioxidant activity.** The TLC with  $\beta$ -carotene spray showed that the purified oat extract (after CC) produced a darker orange color than did the crude extract, suggesting that purified oat extract had better antioxidant activity than did the crude extract. No antioxidant activity was found in the hexane eluant of CC. The dried purified extract had little antioxidant activity, which agreed with the TPC results.

**Identification of chemical components.** The TLC and sprays described in Table 1 were used to tentatively identify the antioxidants present in the modified methanolic extract. Six bands were revealed under UV radiation with  $R_f$  values of 0.93, 0.81, 0.62, 0.53, 0.41 and 0.29. Three of these bands [ $R_f = 0.93$  (A), 0.81 (B) and 0.29 (C)] tested positive with  $\beta$ -carotene spray. These three bands were tested with the sprays, and results are listed in Table 1.

Bands B and C had  $R_f$  values (0.81, 0.29) similar to those found by Duve and White ( $R_f = 0.80, 0.30$ ) (14). Band A ( $R_f = 0.93$ ) had a similar chemical content, but different  $R_f$  than did band B ( $R_f = 0.64$ ) found by Duve and White (14). Perhaps the phenolics were bound by different numbers of chemical groups in the current and previous studies.

**GC and GC/MS identification of phenolic acid composition.** The presence of both ferulic and caffeic acids in the extract was confirmed by both GC and GC/MS.

**Oil storage tests.** No significant differences were found in FAME of any treatments during storage of soybean and cottonseed oils. In addition, the FAME of unsaturated fatty acids dropped little in all treatments by the end of the storage tests. Beginning FAME for soybean and cottonseed oils are shown in Table 3.

TABLE 2

Total Phenolic Content from Each Change of Methanol

Solvent changes	Phenolic content (ppm) <sup>a</sup>
1	15.97
2	7.89
3	4.25
4	1.41
5	0.67
6	0.24
7	0.10
Total	30.53

<sup>a</sup>The phenolics were extracted from 1000 g of oat groats.

TABLE 3

Fatty Acid Composition (relative area %) of Fresh Soybean and Cottonseed Oils

Oils	14:0	16:0	18:0	18:1	18:2	18:3
Soybean	— <sup>a</sup>	11.0	4.2	23.8	53.5	7.8
Cottonseed	1.0	24.1	2.4	17.5	53.9	— <sup>a</sup>

<sup>a</sup><0.01%.

Figure 1 shows the results of cottonseed oil treatments stored at 30°C in the dark for 30 d. The PV of the control oil was significantly higher than PV of all other treatments after 8 d of storage. The treatments containing 0.005, 0.02 and 0.03% were not significantly different from each other until day 18, when the treatment containing 0.005% oat extract was significantly higher in PV than the other treatments containing oat extract. The oil containing 0.03% oat extract had a significantly lower PV than did the oil containing 0.02% oat extract on days 28 and 30. The treatment containing 0.02% TBHQ was not significantly different in PV from the oils containing different levels of oat extract until day 16, after which the treatment containing 0.02% TBHQ maintained a significantly lower PV than all other treatments. The magnitude of the differences between treatments that contained oat extract and the treatment containing 0.02% TBHQ, however, was not great.

When cottonseed oil was stored at 60°C in the dark for 26 d (Fig. 2), the control oil was significantly higher in PV than all other treatments from day 2 on. Until day 4, no significant differences were found among the treatments containing different levels of oat extract and TBHQ. The treatment containing 0.005% of oat extract had a significantly higher PV than treatments containing the other two levels of oat extract and TBHQ after 4 d of storage. From day 18 on, the treatment containing 0.02% oat extract had a significantly higher PV than did the treatments containing 0.03% oat extract and TBHQ; however, the treatment containing 0.03% oat extract had a significantly lower PV than did all other treatments after day 14.

During storage of cottonseed oil at 30°C in the light (Fig. 3), the control treatment was significantly higher in PV than were all other treatments. On day 2, the treatments containing 0.01 and 0.02% oat extract, which were not significantly different from each other, had significantly higher PV than did the treatments containing 0.03% oat extract and 0.02% TBHQ. There was no significant difference between the last two treatments. After day 2, the treatment containing TBHQ had the lowest PV followed by the treatments containing 0.03, 0.02 and 0.01% oat extract, respectively, which were all significantly different from each other.

Storage of soybean oil treatments at 60°C in the dark (Fig. 2) revealed no significant differences among the treatments that contained any level of oat extract or TBHQ. After day 2, the PV of the control oil were much higher than were PV of the rest of the treatments.

Figure 3 shows the results of soybean oil treatments stored at 30°C in the light. After day 4, the treatments that contained oat extract had significantly lower PV than did the control. The treatment containing 0.02% TBHQ

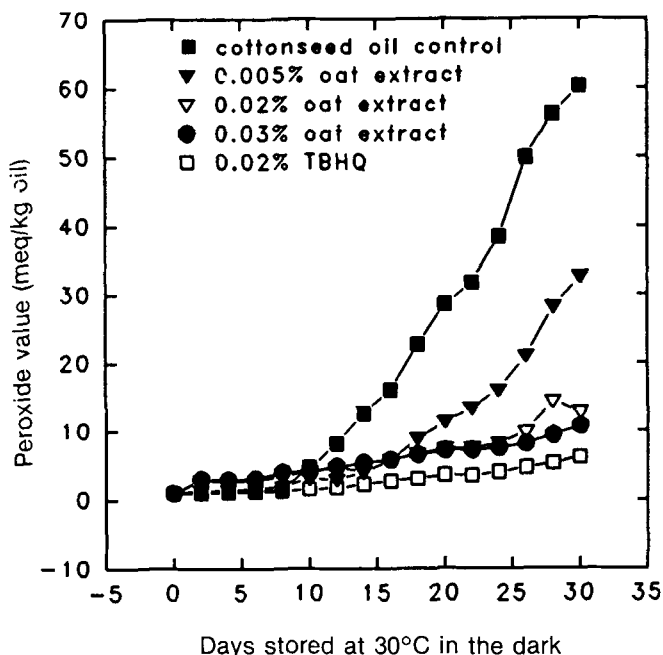


FIG. 1. Peroxide values of cottonseed oil treatments stored at 30°C in the dark. TBHQ = tertiary butyl hydroquinone.

was significantly lower in PV than all other treatments throughout storage. On days 2 and 4, the treatment containing 0.01% oat extract was significantly lower in PV than treatments containing the other levels of oat extract. But by days 6, 8 and 10, no significant differences were found among the treatments that contained different levels of oat extract.

Oat extract was similarly effective in both soybean and cottonseed oils, even though the degree of unsaturation in soybean oil was higher than in cottonseed oil (Table 3). The oat antioxidant tended to give better protection in both soybean and cottonseed oils in the dark at accelerated room temperature (60°C) than did TBHQ (Fig. 2).

At 30°C in the light and 60°C in the dark, the oat extract at 0.005% was much less effective in cottonseed oil than at 0.02 and 0.03%. Because no differences were found within soybean oils containing oat extract at levels of 0.01, 0.02 or 0.03% and cottonseed oil containing 0.02 or 0.03% oat extract, perhaps 0.01% oat extract was the minimum amount needed for maximum effectiveness.

In all storage tests, the induction periods of the oils containing oat extract at all levels or TBHQ were much longer than was the induction period of the control. Some antioxidant treatments had not even reached the end of their induction period by the end of the storage test. For example, at 60°C in the dark (Fig. 2), the cottonseed oil control reached the end of its induction period in 2 d, whereas the treatments containing 0.03% oat extract or TBHQ had not reached the end of the induction period after 26 d of storage. The treatments containing 0.005 and 0.02% oat extract had induction periods of 8 and 18 d, respectively. Other tests showed similar results.

The results in the current experiments are different from those of Duve and White (14) who cited no significant differences in PV among soybean oil treatments that con-

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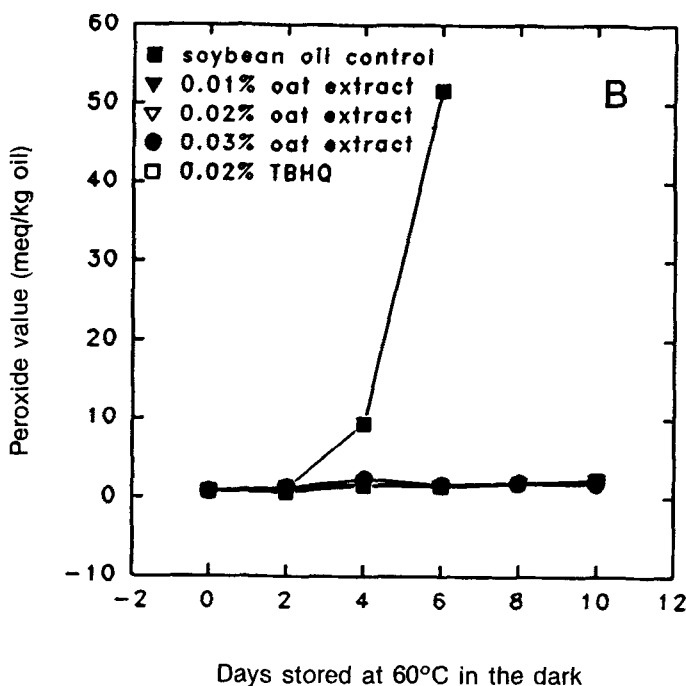
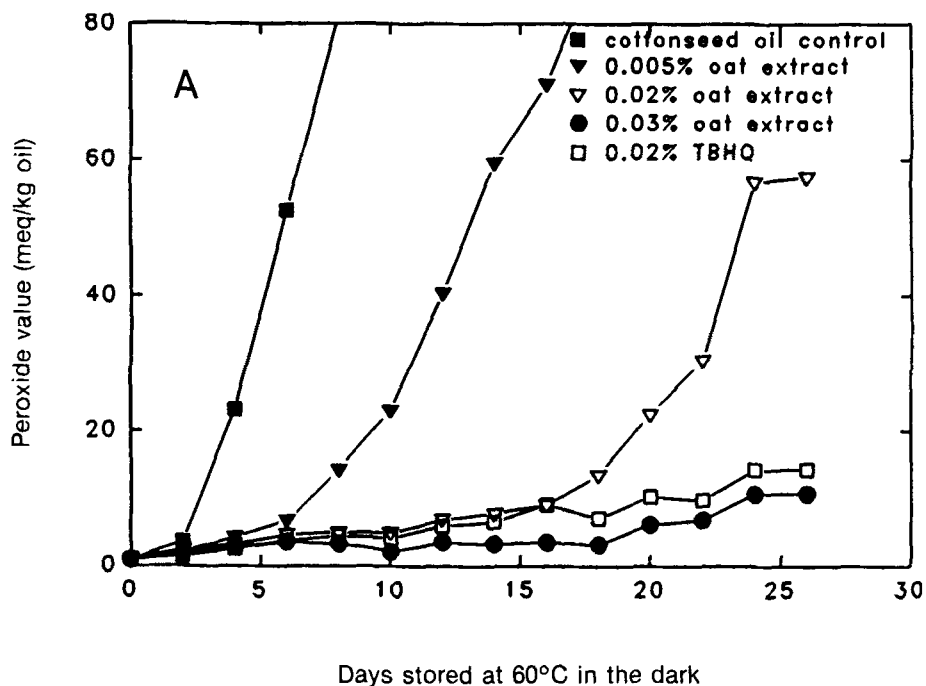


FIG. 2. (a) Peroxide values of cottonseed and (b) soybean oil treatments stored at 60°C in the dark. Abbreviation as in Figure 1.

tained oat extract and a control stored at 60°C for 20 d. But they did note some effectiveness of oat extract in soybean oil stored at 32°C in the dark for 80 d. The significant improvement of the oat extract in the current experiments is likely due to the higher purity of the oat extract

obtained by the modified extraction procedure and because the amount of extract added in the current tests was based on total phenolic content and not just weight of the extract.

*Emulsion storage tests.* The initial FAME of soybean

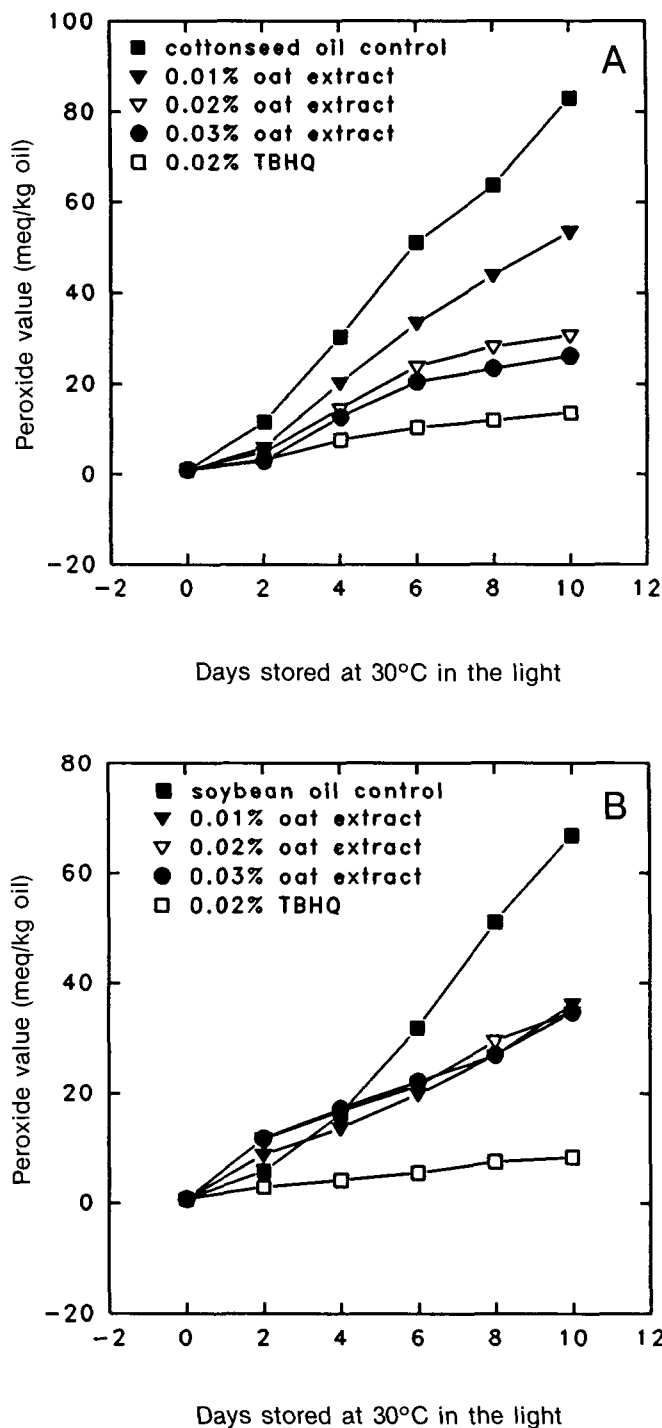


FIG. 3. (a) Peroxide values of cottonseed and (b) soybean oil treatments stored at 30°C in the light. Abbreviation as in Figure 1.

and cottonseed oils used in the emulsions were the same as those for the pure oils (Table 3). No significant differences in FAME were noted among treatments during storage, and the decreases in unsaturated fatty acids were small by the end of the storage tests, so these data are not shown.

During 6 d of storage at 30°C in the light, the treatments that contained oat extract or TBHQ had signifi-

cantly lower PV than did the control (Fig. 4). In general, no significant differences were observed among the treatments that contained any level of oat extract throughout the study. The treatment containing TBHQ had a significantly higher PV than did the treatments containing any level of oat extract on day 6.

When stored at 60°C in the dark (Fig. 5), the treatments containing any level of oat extract were not significantly different from each other until the last day of storage, at which time the treatment containing 0.03% oat extract had a significantly lower PV than did the treatments with the other two levels of oat extract. The treatment containing TBHQ had a significantly higher PV than did the treatments containing any level of oat extract throughout the study. On day 6, the PV of the treatment containing TBHQ was much higher than any of the treatments with oat extract. The PV of the control was significantly higher than were PV of the rest of the treatments throughout the study.

During storage of soybean oil emulsions at 30°C in the light (Fig. 4), the treatments containing added antioxidants had significantly lower PV than did the control oil. On day 2, no significant differences were found among the treatments containing different levels of oat extract and TBHQ. On days 4 and 6, the treatments containing different levels of oat extract were not significantly different from each other, but they had significantly higher PV than did the treatment containing TBHQ. On day 8, the treatments containing 0.03% oat extract and TBHQ, which were not significantly different from each other, had significantly lower PV than did the treatments containing 0.01 and 0.02% oat extract. Practically speaking, however, the differences among the antioxidant-treated emulsions probably were not important. On day 10, no significant differences were found among the treatments containing different levels of oat extract and TBHQ.

During 10 d of storage at 60°C in the dark (Fig. 5), the soybean oil treatments with all levels of oat extract and TBHQ had significantly lower PV than did the control. On day 2, treatments containing the different additives were not significantly different from each other, although, by day 4, the treatments containing any level of oat extract had significantly lower PV than did the treatment containing TBHQ. By day 6, the treatment containing 0.01% oat extract had a significantly higher PV than did the treatments containing the other two levels of oat extract, but all the treatments with oat extract had significantly lower PV than did the treatment containing TBHQ. On days 8 and 10, the treatment containing 0.03% oat extract had a lower PV than did the treatments containing the other two levels of oat extract, which were not significantly different from each other. Under conditions of 60°C storage in the dark, the oat antioxidants were far superior to TBHQ at reducing oxidation of the soybean oil emulsions.

In general, oat extract was more useful as an antioxidant in emulsions than was TBHQ, especially at 60°C storage in the dark. This effect may be because there are several components in oat extract giving a range of solubilities in different systems, thus allowing some compounds to be more soluble at the oil/water interphase in the oil and some more soluble in the water. In contrast, TBHQ, a single compound, was more soluble just in the oil phase. These results agree with those of Musher (3),

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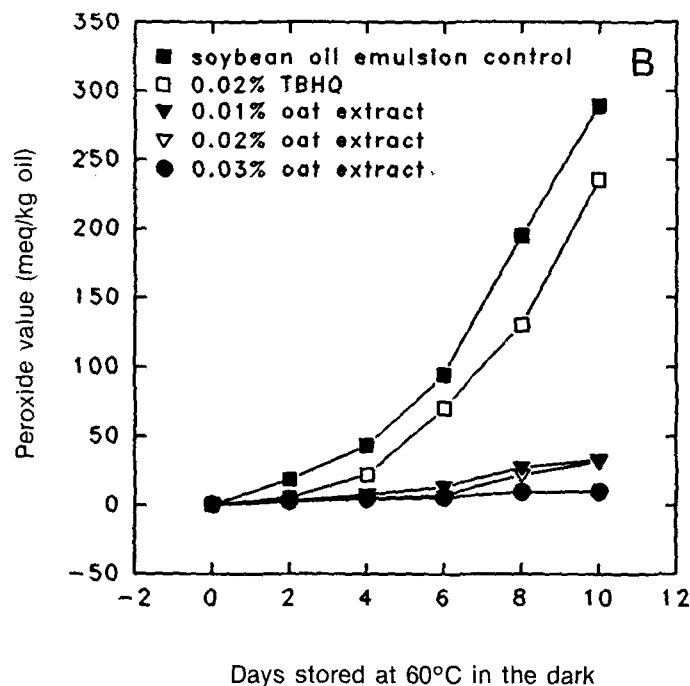
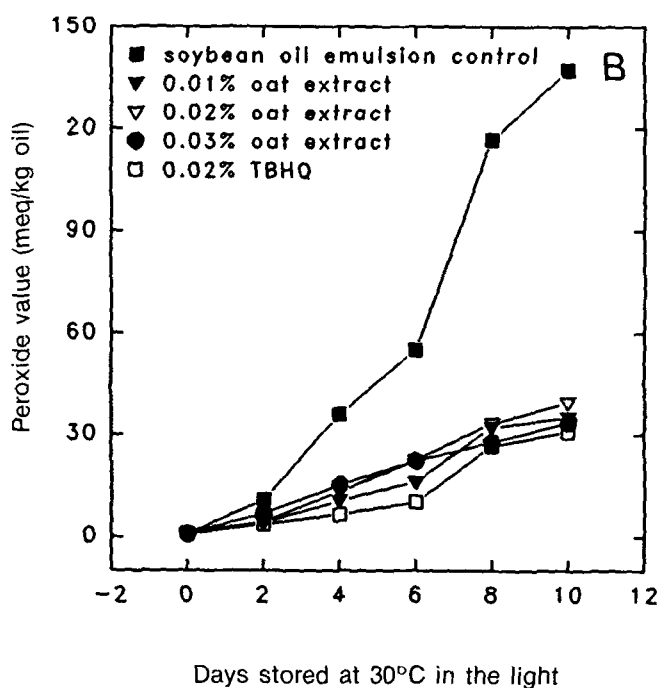
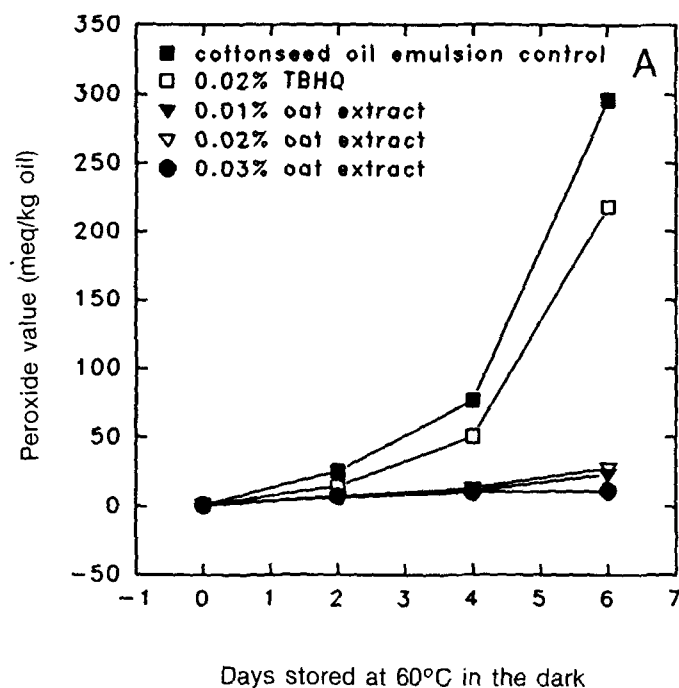
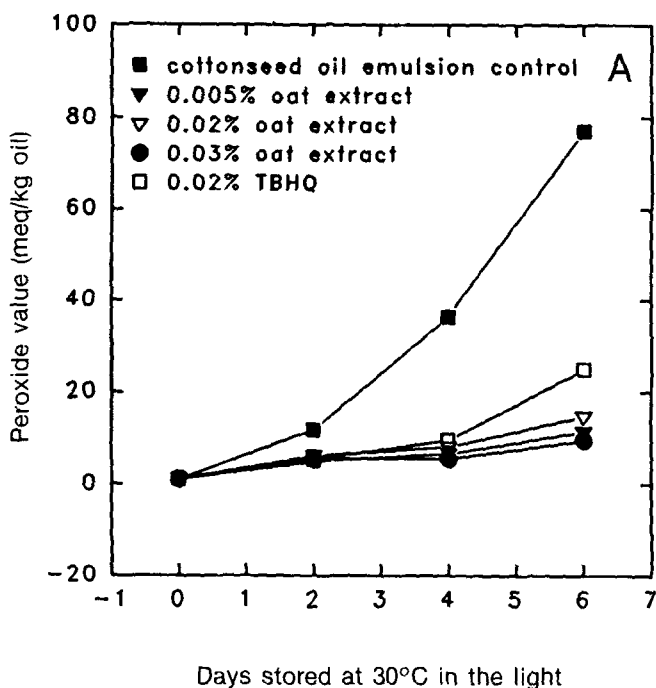


FIG. 4. (a) Peroxide values of cottonseed and (b) soybean oil emulsions stored at 30°C in the light. Abbreviation as in Figure 1.

FIG. 5. (a) Peroxide values of cottonseed and (b) soybean oil emulsions stored at 60°C in the dark. Abbreviation as in Figure 1.

who reported that an aqueous extract of oat was markedly effective in protecting oil and fat emulsions. Chipault *et al.* (33) also found that most of 32 spices they tested in a ground form were more effective against oxygen absorption in lard emulsions than in plain lard.

**Quantity of extract needed.** The amount of oat needed to protect oil, when added at a level of 0.01% extract, based on actual phenolic content, was 3.3 parts oat to 1

part oil: a great quantity of oat! These figures are based on obtaining 30 mL of oat extract from 1 kg of oats, with the extract having a total phenolic content of 0.1% (wt/vol). Obviously, this amount of oat is too great to provide an economical source of natural antioxidants unless the antioxidant extraction is coupled with the production of other products from the same oats. For example, antioxidants may be obtained after the extraction of oil from

high-oil-containing oats. The extract from oat hulls, which contains phenolic antioxidants and  $\Delta^5$ -avenasterol (14,34,35), may be another way to lower the cost of oat antioxidants. Oat hulls make up about 30% (wt/wt) of whole oats.

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#### REFERENCES

- Haigh, R., *Food Chem. Toxic.* 24:1031 (1986).
- Musher, S., U.S. Patent 2,075,824 (1937).
- Musher, S., U.S. Patent 2,176,026 (1939).
- Musher, S., U.S. Patent 2,176,027 (1939).
- Musher, S., U.S. Patent 2,181,765 (1939).
- Daniels, D.G.H., and H.F. Martin, *Nature* 191:1302 (1961).
- Daniels, D.G.H., and H.F. Martin, *J. Sci. Food Agric.* 14:385 (1963).
- Daniels, D.G.H., and H.F. Martin, *Ibid.* 18:589 (1967).
- Daniels, D.G.H., and H.F. Martin, *Ibid.* 19:710 (1968).
- Collins, F.W., *J. Agric. Food Chem.* 37:60 (1989).
- Collins, F.W., D.C. McLachlan and B.A. Blackwell, *Cereal Chem.* 68:184 (1991).
- Supova, J., J. Pokorny and G. Janicek, *Sb. Vys. Sk. Chem. Technol. Praze. Oddil Fak. Potravinny Technol.* 3:525 (1959).
- Chang, S.S., B. Ostric-Matijasevic, O.A.-L. Hsieh and C.-L. Huang, *J. Food Sci.* 42:1102 (1977).
- Duve, K.J., and P.J. White, *J. Am. Oil Chem. Soc.* 68:365 (1991).
- AOAC *Official Methods of the Analytical Chemists*, Association of the Official Analytical Chemists, Washington, D.C., 1984.
- Kurechi, T., M. Aizawa and A. Kunutgi, *J. Am. Oil Chem. Soc.* 60:1878 (1983).
- Hamm, D.L., E.G. Hammond, V. Parvanah and H.E. Snyder, *Ibid.* 42:920 (1965).
- Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1989.
- Hammond, E.G., and W.R. Fehr, in *Biotechnology for the Oils and Fats Industry*, edited by C. Ratledge, P. Dawson and J. Ratray, American Oil Chemists' Society, Champaign, 1985, p. 89.
- Pratt, D.E., and E.E. Miller, *J. Am. Oil Chem. Soc.* 61:1664 (1984).
- Taga, M.S., E.E. Miller and D.E. Pratt, *Ibid.* 61:928 (1984).
- Barton, G.M., R.S. Evans and J.A.F. Gardner, *Nature* 170:249 (1952).
- Reio, L., *J. Chromatogr.* 1:338 (1958).
- Hollar, N.S., A Study of Lipid Autoxidation of Soy Protein Isolates, Ph.D. Thesis, Purdue University, West Lafayette, 1974.
- Roux, D.G., and H.E. Maihs, *J. Chromatogr.* 4:65 (1953).
- Swain, T., *Biochem. J.* 53:200 (1953).
- Swain, T., in *Data for Biochemical Research*, Vol. 1, edited by R.M.C. Dawson, D.C. Elliott, W.H. Elliott and K.M. Jones, Oxford University Press, New York, 1969, pp. 558-562.
- Bailey, R.W., *Ibid.* pp. 539-544.
- Nicholson, R.L., Production of Xylamases by *Verticillium Albo-Atrum*, M.S. Thesis, University of Maine, Orono, 1967.
- Pridham, J.B., *Anal. Chem.* 28:1967 (1956).
- Pometto, A.L., and D.L. Crawford, *Methods Enzymol.* 161:175 (1988).
- SAS, SAS Institute, Inc., Cary, 1985.
- Chipault, J.R., G.R. Mizuno and W.O. Lundberg, *Food Technol.* 9:443 (1955).
- White, P.J., and L.S. Armstrong, *J. Am. Oil Chem. Soc.* 63:525 (1986).
- Appelqvist, L.-A., and S. Helmersson, *INFORM* 4:528 (1993).

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