Novel Natural Antioxidant for Stabilization of Edible Oil: The Ajowan (*Carum copticum*) Extract Case

D. Bera[a], D. Lahiri[a], and A. Nag[b,*]

[a]Rural Development Center and [b]Chemistry Department,
Indian Institute of Technology, Kharagpur-721302, India

ABSTRACT: An inexpensive, natural antioxidant was developed for stabilizing flaxseed and bahera oils, which are readily available and consumed in large quantities for their EFA in India, Asia, and also parts of the Western world. With the incorporation of soluble ajowan (*Carum copticum*) extract, as ajowan powder, into the oils, their oxidation could be prevented. After storage of the oils for a year in the presence of antioxidant, the odor, taste, and chemical properties of the oil were the same.

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KEY WORDS: Ajowan extract, ajowan powder, antioxidants, bahera oil, flaxseed oil, linolenic acid [18:3(9,12,15)].

Production of functional foods for the prevention of diseases (1–4) such as obesity, diabetes, and heart diseases is becoming more important. But lipid peroxidation lowers the nutritive value of food (4) and adversely affects its flavor and taste. Lipid oxidation must be suppressed to maintain the safety and effectiveness of food. Functional foods containing α-linolenic acid [18:3(9,12,15)] cannot be formulated without the simultaneous incorporation of an acceptable antioxidant, because linolenic acid is prone to rapid oxidation both in storage and in the human body (3). Generally, food oxidation can be prevented by the inclusion of synthetic antioxidants such as BHA, BHT, ethoxyquin, and propyl gallate (PG), but their safety has been questioned (5). Hence, there is a need to identify safer and more efficient natural antioxidants for use in the food industry. Recently, Nag (6) showed that *Capsicum* extract can be used as a natural antioxidant to stabilize edible oils.

Owing to their high content of EFA (Table 1), bahera (*Terminalia bellirica roxburghii*) and flaxseed (*Linum usitatissimum*) oils are used in large amounts as edible oils in India, Asian countries, and some parts of the Western world. These oils are very prone to oxidation (6), making preservation over a long time difficult. Here we report on studies regarding the oxidation and stability of these two oils in the absence and presence of an inexpensive, natural antioxidant, ajowan extract (AE); an alternative name for ajowan is *Carum copticum*.

MATERIALS AND METHODS

Bahera oil was extracted from bahera seed and flaxseed oil was extracted from husk-free ground flaxseed with *n*-hexane (b.p. 60–65°C) in a Soxhlet apparatus. FA in the oil were analyzed as follows: 100–200 mg of oil and 10 mL of 0.7% methanolic HCl were refluxed on a water bath at 70°C for 2 h. The content was cooled, 0.5 mL of water was added, and the ester was separated by washing with petroleum ether (to remove excess acid) and by drying with anhydrous sodium sulfate. The analysis of methyl esters was performed by measuring the peak areas from GLC (Model AIMIL-UNCON, series 5700; Hewlett-Packard, Mississauga, Ontario, Canada), injection temperature, 250°C; column temperature, 240°C; silica capillary 15% FEAP column (0.025 mm × 60 m; flow rate, 40 mL/min; carrier gas, nitrogen; Hewlett-Packard). FA analyses of both oils are listed in Table 1.

Oils were oxidized by holding them in a thermostatically controlled oil bath, from 30 to 200°C, for 2 min. Then readings were taken. Air from an air cylinder (cylinder pressure, 1.7 kg/cm^2) was passed through the oil at a rate of 2 mL/min. Oil samples were then taken over time for determination of PV and thiobarbituric acid (TBA) values (7). The peroxides formed were measured iodometrically by a standard food analysis method, and the TBA test was based on the color reaction of TBA with malondialdehyde (mg/kg) in the sample (7). The experiment was replicated three times, and the average values were reported. The percentage error was ±0.05.

For determination of PV values at different temperatures, the oil was kept in the thermostated bath at a fixed temperature for different time intervals, then withdrawn for analyses.

Commercial ajowan powder [Datta Company, West Bengal, India; 1 kg for 120 Rs (approx. $3 U.S.)] was purchased from the local market, washed three times with distilled water,

TABLE 1
Properties of Flaxseed and Bahera Oils[a]

	Flaxseed oil	Bahera oil
Refractive index at 20°C	1.2	1.1
Density at 20°C (g/mL)	0.889	0.845
Palmitic acid, $C_{16:0}$	6.0%	21.5%
Stearic acid, $C_{18:0}$	7.1%	7.9%
Oleic acid, $C_{18:1}$	22.2%	57.1%
Linoleic acid, $C_{18:2}$	14.2%	7.8%
Linolenic acid, $C_{18:3}$	50.4%	5.7%
FFA (mg/g)	2.0	1.62
PV (mg/g)	5.2	5.09
Saponification value (mg/g)	220.0	170
Iodine value (g/100 mg)	82.0	80

[a]Flaxseed, *Linum usitatissimum*; bahera, *Terminalia bellirica roxburghii*.

*To whom correspondence should be addressed.
E-mail: ahinnag@chem.iitkgp.ernet.in

oven-dried at 70°C for 4 h, and sieved to pass a 200 mesh. Antioxidant was extracted from ajowan powder with ethanol (1:15, wt/vol). This extracted antioxidant was dissolved in minimum weight of the oil (0.025 g/g of oil). This calculated amount was used for experimental purposes. All the experimental data were three replicates, and the percentage error was ±0.5.

The alcoholic extract from ajowan was subjected to column chromatography. The first fraction (about 50%) was eluted from the column by passage of a petroleum ether/ethyl acetate mixture (20:1). The structure of the solid compound remaining after evaporation of the solvent, 5-methyl-2-isopropyl phenol, or thymol, was characterized by IR and NMR: IR (range 200–4000 cm^{-1}, KBr); 3338 (phenolic OH str.), 2961 (aromatic C–H str.), 2872, 2749, and 2604 (aliphatic C–H str.), 1154, 1091, and 1002 (C–C aliphatic str.), and 1246 (C–O str.) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, δ): 7.09 (*d*, 1H, *J* = 7.88 Hz,

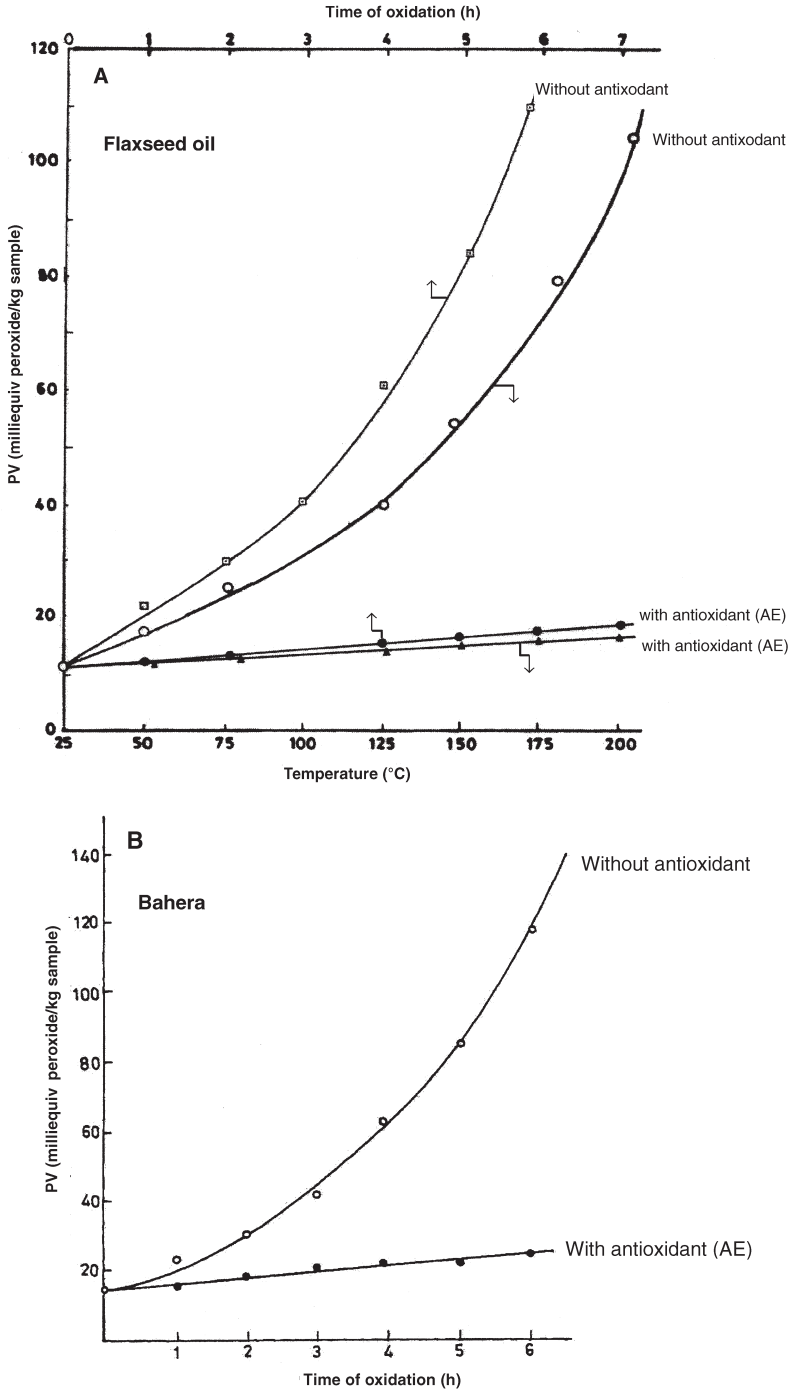

FIG. 1. PV of flaxseed oil at (A) different temperatures and different times in the presence of air at 30°C: open symbols (○, □) without antioxidant, closed symbols (▲, ●) with antioxidant, where the antioxidant is ajowan extract (AE); and of (B) bahera oil at different times in the presence of air: (○) without AE, (●) with AE.

Ar-3H), 6.73 (*d*, 1H, *J* = 3.86 Hz, Ar-4H), 6.58 (*s*, 1H, Ar-6H), 4.6–4.8 (*bs*, 1H, Ar-OH), 3.13–3.20 [*m*, 1H, $(CH)_3CH$)], 2.28 (*s*, 3H, Ar-CH_3), 1.25 [*d*, 6H, *J* = 6.86 Hz, $(CH_3)_2CH$–]. ^{13}C (200 MHz, $CDCl_3$, δ): 20.781, 22.637, 26.701, 116.050, 121.661, 126.207, 131.376, 136.538, 152.479.

The other nonvolatile oil fractions were eluted by a solvent mixture of ethyl acetate and petroleum ether (1:40), indicating that the mixture contained four compounds (confirmed by TLC), which did not show any antioxidant properties. The first fraction was collected for experimental trials and was used with bahera and flaxseed oils as an antioxidant to evaluate the oxidation properties of the oils.

RESULTS AND DISCUSSION

The FA values of bahera and flaxseed oils (Table 1) reflect that the oils contain high amounts of unsaturated FA (86.8% for flaxseed oil, 70.6% for bahera oil). These are more prone to peroxide formation by reaction with oxygen in air. This was experimentally proved by the sharp increase in PV of bahera and flaxseed oils (Figs. 1A, B) at different times in the absence of AE, indicating that both oils are highly oxidizable. In the presence of antioxidant (AE, antioxidant/oil ratio = 1:40 w/w), which is sufficient to prevent peroxide formation of unsaturated oils), the PV of bahera and flaxseed oils remained relatively constant during oxidation. Similarly, TBA values of the oil samples remained constant with time in the presence of AE (Fig. 2). There was also a sharp rise in PV with increasing temperature (Fig. 1A). But in the presence of AE (1:40 w/w), the PV remained relatively constant with increasing temperature (Fig. 1A). Thus, the incorporation of oil-soluble AE (antioxidant) inhibited the rate of oxidation to such a degree that there was almost no rise in TBA values (Fig. 2).

Phenolic substances (acting as antioxidants) function as free radical acceptors and can terminate free radicals at the initiation stage (8). Adegoke *et al.* (9) concluded that hindered phenolics such as BHA, BHT, TBHQ, EQ, and tocopherols as well as polyhydroxyphenolics such as PG are primary antioxidants,

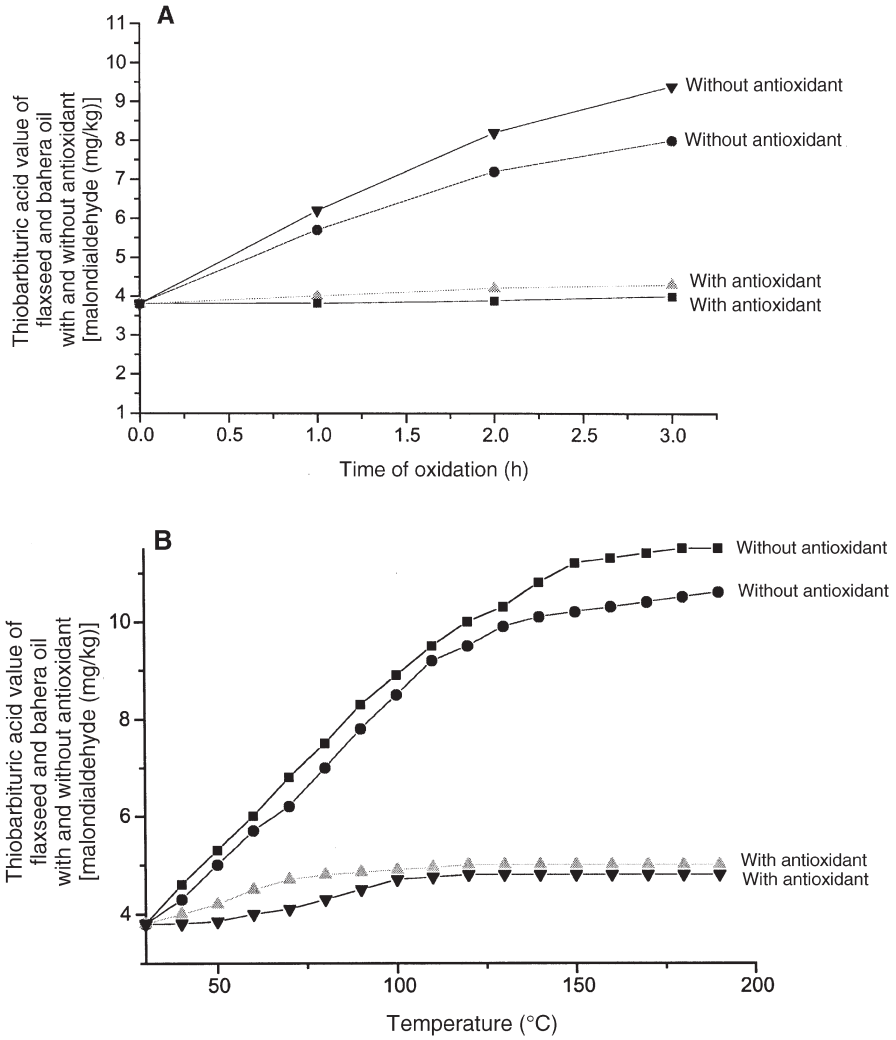

FIG. 2. Thiobarbituric acid value of flaxseed oil and bahera oil at (A) different times and (B) different temperatures in the presence of air. (A) Flaxseed oil: (■) without AE, (●) with AE. Bahera oil: (△) without AE, (▼) with AE. (B) Flaxseed oil: (●) without AE, (▼) with AE. Bahera oil: (■) without AE, (▲) with AE. For abbreviation see Figure 1.

which either delay or inhibit the initiation step by reacting with a lipid free radical, or inhibit the propagation step by reacting with the peroxy or alkoxy radicals. As the first fraction of AE contains thymol, an active principle with a phenolic group in its structure, AE actively prevents (does not initiate or propagate) further oxidation of the glycerides.

The properties of the oil with antioxidant, such as changes of FFA, PV, iodine value, saponification value, and TBA value, were determined every month, and we found that there was a slight change. The experiments were continued for the whole year. We may conclude that oils with added AE did not deteriorate during storage for 1 yr.

We served salad prepared with this oil plus AE to 50 persons. They found that the taste of the oil was unobjectionable and had no ill effects (6). A lack of animal testing facilities prohibited enquiries into the biological value of oils containing AE.

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