mained essentially the same. Additional work is needed to evaluate the ease of processing the crude oil obtained with limited volumes of hexane, and the nutritive value of the resulting meal for feeding.

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Oxidative Stability of Jojoba Wax

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The rates of autoxidation of crude, bleached and stripped jojoba wax were determined under conditions of accelerated oxidation (98 C). Oxidation of the raw yellow wax had a long induction period (50 hr) compared with the bleached wax (10–12 hr) or stripped wax (2 hr). These differences indicate the presence of a natural antioxidant in the crude wax. Addition of 0.02% butylated hydroxytoluene or butylated hydroxyanisole to the bleached wax restored and even improved its stability. Autoxidation of jojoba wax was also studied at room temperature. In the presence of light and air, the activity of the natural inhibitor was rapidly lost.

The jojoba shrub (*Simmondsia chinensis* [Link] Schneider) (1,2) yields dark brown, nut-like seeds from which a bright yellow liquid wax may be extracted (3,4). The major constituents of the wax are straight-chain esters of C20 and C22 δ-9 mono-unsaturated alcohols and carboxylic acids (5,6). The wax is free of rancidity (7) and stable to oxidation (8), but this property has not been investigated thoroughly. The growing interest in the development of this shrub as a cultivated oilseed crop to replace sperm whale oil (7) led us to study the stability of the wax to autoxidation. To satisfy requests by jojoba wax consumers in the cosmetic industry, we performed accelerated oxidation of the original crude wax as well as of the bleached and stripped waxes. We also determined the ability of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) to protect bleached wax against oxidation.

EXPERIMENTAL PROCEDURES

Materials. Crude jojoba wax was obtained from the Apache Marketing Cooperative Association, San Carlos, Arizona. Bleached wax was prepared by treating the crude wax twice with 1% w/w commercial bleaching earth at 80 C. Stripped wax was obtained from crude wax by distilling off and discarding the fraction boiling up to 170 C at 1 mm Hg. BHT and BHA, purchased from Sigma Chemical Co., St. Louis, Missouri, were first dissolved in a small amount of wax by gentle warming. Then wax was added to obtain the required concentration.

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Apparatus. Oxidation was done in 250 ml gas washing bottles. Two hundred g of wax were used in each run. The bottles were immersed in a thermostatically controlled insulated water bath at 98 C. The accelerated oil oxidation procedure is described in AOCS tentative method Cd 12-57 (9). The refractive index of the wax was determined with an Abbe Refractometer, Bellingham and Stanley Model 60/HR, at 60 C.

Peroxide determination. The hydroperoxide content of 50 to 1000 mg of wax was determined by AOCS official method Cd 8-53 (10) using 0.001 and 0.01 N sodium thiosulfate solutions.

RESULTS AND DISCUSSION

The initial peroxide value of the crude jojoba wax was 17 meq/kg. After stripping or bleaching the wax, this value fell to almost zero. The changes in the peroxide values of the wax samples during accelerated oxidation are shown in Figure 1. In spite of its high initial peroxide value, the crude wax had a long induction period of

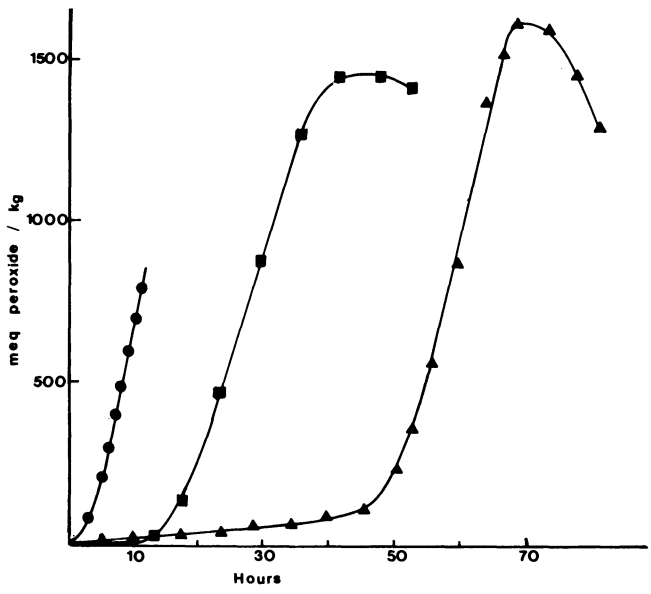

FIG. 1. Oxidation of jojoba wax by air under accelerated conditions (98 C). ▲, Crude wax; ●, stripped wax; ■, bleached wax.

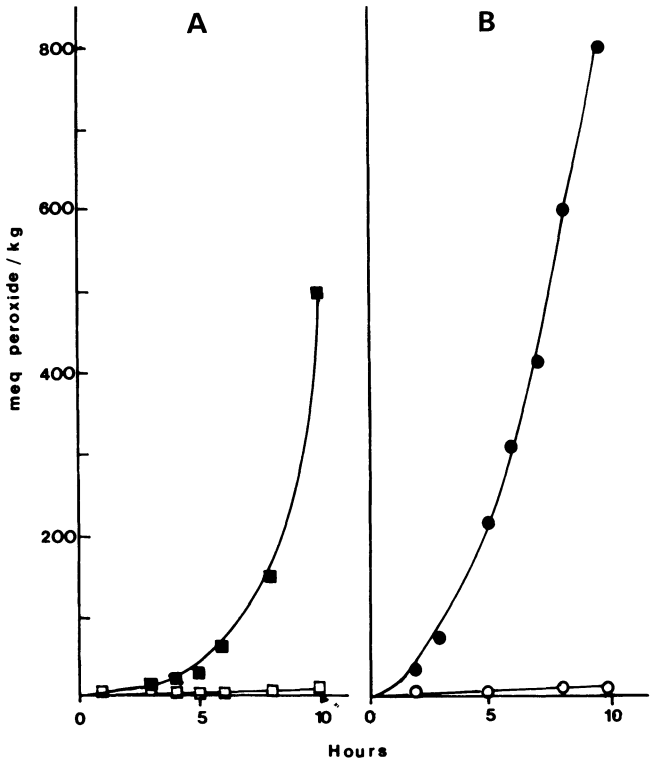

FIG. 2. Effect of addition of forerun on oxidation rate. (A) Addition to bleached wax. ■, Bleached wax; □, bleached wax + forerun. (B) Addition to stripped wax. ●, Stripped wax; ○, stripped wax + forerun.

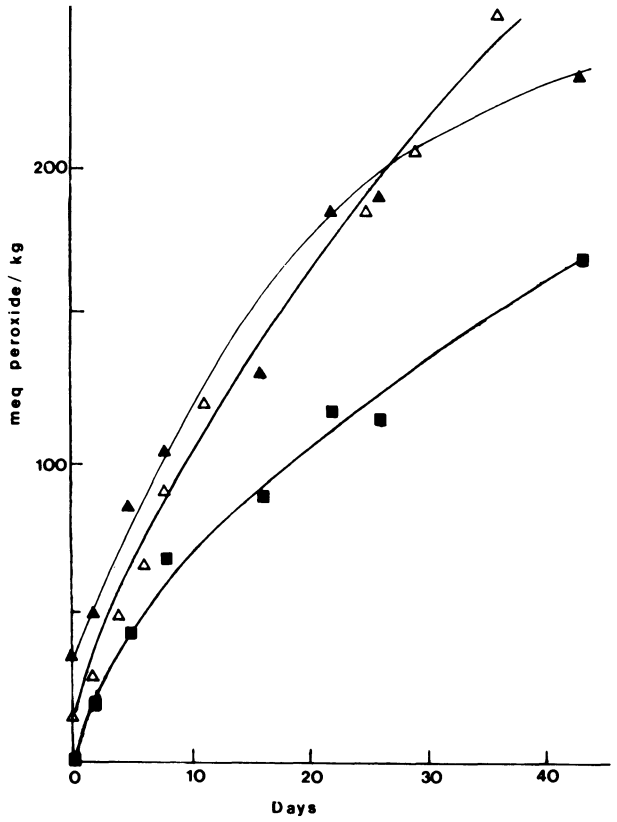

FIG. 3. Effect of light and air on oxidation rate. ▲, Crude wax, direct sunlight; ■, bleached wax, direct sunlight; △, crude wax, indoor sunlight.

TABLE 1

Induction Periods of Different Waxes during Accelerated Oxidation at 98 C

Wax sample	Method	
	Oxidation curve[a]	Refractive index curve[b]
Crude wax[c]	45	45
Stripped wax[c]	2	—
Bleached wax[c]	12	—
Bleached wax + 0.02% BHT	50	52
Bleached wax + 0.02% BHA	85	85

[a]Oxidation curve: meq peroxide/kg wax per hr.

[b]Refractive index curve: change of refractive index per hr.

[c]Values derived from Fig. 1.

45–50 hr. In contrast, the bleached and stripped waxes had short induction periods of 12 and 2 hr, respectively, in spite of their initial zero peroxide values. These results suggest the presence in the crude wax of a natural antioxidant that is lost in the bleaching and stripping processes.

To identify this inhibitor in crude jojoba wax, the bleaching earth recovered after bleaching was extracted with petroleum ether, chloroform and ethanol. The extract was tested for antioxidant activity on bleached wax by accelerated oxidation tests. The oxidation rates were increased. Thus, the stabilizing factor may have decomposed while adsorbed on the bleaching powder and exposed to air and light.

The forerun from the distillation (the fraction boiling up to 170 C) was tested similarly. This fraction of the wax did contain an active stabilizing factor, because the oxidation of bleached and distilled wax (Fig. 2) was inhibited by the addition of the forerun.

In an attempt to isolate the stabilizing factor, a solution of the crude wax in petroleum ether was extracted with acetonitrile according to an IUPAC method for extraction of antioxidants (11). UV absorbtion spectra indicated a phenolic derivative (λ = 267 nm), mass spectrometry (M^+163) and NMR(δppm 4.1;5.3) results suggesting that the substance may be an allylic derivative of hydroxy toluene. According to Miwa (12) the antioxidant is not a tocopherol.

When freshly bleached jojoba wax was kept in the dark at room temperature, the peroxide value remained in the low range of 0–0.5 meq/kg for several months. However, on the bench in an ordinary transparent glass bottle, the wax acquired a peroxide value of 70 meq/kg within six to seven weeks. The changes in the peroxide value of the crude wax also were followed when the wax was stored in an open glass beaker at room temperature in daylight (in the laboratory) and under direct sunlight (on the roof). The stability of the bleached wax also was studied under direct sunlight. The results (Fig. 3) show that, in the presence of light and air, the activity of the natural inhibitor is rapidly lost, and a peroxide value of 150–250 meq/kg was obtained within a few weeks.

The possibility of protecting the wax with synthetic antioxidants was tested by submitting bleached jojoba

wax containing 0.02% BHT or BHA to accelerated oxidation. The induction periods of oxidation, as determined from the oxidation curve and the refractive index curve (Table 1), proved BHT an effective antioxidant comparable to the natural antioxidant present in crude wax, while BHA was found superior even to it.

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Adulterated Butterfat: Fatty Acid Composition of Triglycerides and 2-Monoglycerides

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Beef tallow and cottonseed oil were mixed with a pure butterfat in the ratios of 2%, 4% and 6% to obtain admixtures of beef tallow with butterfat and cottonseed oil with butterfat. The hydrolysis of individual triglycerides was carried out using the lipase to obtain 2-monoglycerides. The results indicated that butterfat had a higher percentage of C14:0 and C16:0 acids than found in the triglycerides and 2-monoglycerides of beef tallow and cottonseed oil.

Beef tallow contained a higher proportion of C18:0 and C18:1 acids than butterfat and cottonseed oil triglycerides or 2-monoglycerides. Cottonseed oil had a higher percentage of C18:2 acid located in triglyceride or 2-monoglyceride than found in butterfat or beef tallow triglycerides and 2-monoglycerides.

The analysis of the samples of butterfat containing 2%, 4% and 6% beef tallow revealed that the addition of beef tallow to butterfat affected the fatty acid composition of butterfat triglycerides and 2-monoglycerides with C18:0 and C18:1 acids; the effect was increased with increasing percentages of beef tallow.

The addition of cottonseed oil to butterfat in the ratios of 2%, 4% and 6% affected the fatty acid composition of butterfat triglycerides and 2-monoglycerides. It was found that both C18:1 and C18:2 increased as the added cottonseed oil percentages increased.

Several trials were carried out to prove the purity of butterfat. The addition of beef fat less than 5% cannot be detected with certainty and may be missed. The presence of higher percentages will almost always be indicated by the melting point of the crystals deposited from ether solution (1).

TLC separation of butterfat into long and short chain triglycerides, followed by a selective lipolysis of each fraction with pancreatic lipase to determine the C16:C18 ratio in position 2 of the triglycerides of each band, could detect adulteration of butter even with low amounts (5%) of beef or foreign fats (2). The addition of small amounts of pig or buffalo fat to cow or buffalo ghee results in the appearance of an extra peak located at high temperature in the melting and crystallization curves as determined by differential scanning calorimetry. Ghee adulterations with these animal fats at levels down 5% are clearly seen in crystallization diagrams (3).

The fatty acid distribution in the triglycerides of milk and other animal fats can be determined by use of the specificity of pancreatic lipase for cleaving the fatty acids esterified in the 1 and 3 positions of the glycerol. It was found that weight percents of fatty acid composition of original triglycerides and monoglycerides formed of normal milk were C14:0, 12%, 20%; C16:0, 38%, 40%; C18:0, 14%, 8.7%; C18:1, 23%, 15%; and C18:2+3, 4.0%, 1.9%, respectively. The fatty acid compositions of original triglycerides and monoglycerides found in steer depot fat were C14:0, 4.6%, 8.8%; C16:0, 29%, 16%; C18:0, 24%, 14%; C18:1, 36%, 54%; and C18:2+3, 3.0%, 1.2%, respectively (4).

On the other hand, the distribution of fatty acids among the primary and secondary positions of the triglycerides of various species of plants demonstrated that certain fatty acids occupy specific positions on the triglyceride molecule. Palmitic and stearic acids were found to be esterified predominantly at the primary positions, and the fatty acids having a chain length of greater than 18 carbon atoms are also esterified predominantly at the primary positions (5).

Pancreatic lipase digests some classes of milk triglycerides more rapidly than others. It is concluded that milk lipase did not exhibit intramolecular specificity when glyceryl 1-palmitate 2,3 dibutyrate was the substrate (6,7). Palmitic acid showed a definite tendency to

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