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Oxidative Stability of Algal Oils As Affected by Their Minor Components

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Algal oils, namely, arachidonic acid single-cell oil (ARASCO), docosahexaenoic acid single-cell oil (DHASCO), and a single-cell oil rich in both docosahexaenoic acid and docosapentaenoic acid (OMEGA-GOLD oil), were evaluated for their oxidative stability, as such and after stripping of their minor components, in the dark at 60 °C and under fluorescent light at 27 °C. Several analytical methods were used to assess the oxidative stability of these oils. Oil extracts were also investigated for their scavenging of 1,1-diphenyl-2-picrylhydrazyl radical by a spectrophotometric method and by measuring their total phenolic contents. The results indicated that minor oil constituents play a major role in their oxidative stability in the dark as well as under fluorescent light. The stability of the oils was dictated by their fatty acid composition, total tocopherols, and the type of pigment present. DHASCO contained a significant level of natural radical scavengers and phenolic compounds that contributed to its higher stability compared to the ARASCO and OMEGA-GOLD oils. Thus, the importance of minor components in the stability of the oils examined was demonstrated.

KEYWORDS: Algal oils; autoxidation; conjugated dienes; 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical; headspace volatiles; high-performance liquid chromatography; photooxidation; oxidative stability; tocopherol content; thiobarbituric acid reactive substances (TBARS)

INTRODUCTION

It is well recognized that dietary factors influence human health status and life quality. Algal oils produced from unicellular organisms (single cell) such as arachidonic acid (ARA) single-cell oil (ARASCO), docosahexaenoic acid single-cell oil (DHASCO), and a single-cell oil rich in both docosahexaenoic acid (DHA) and docosapentaenoic acid (n-6 DPA) (OMEGA-GOLD oil) are commercially available. Findings on possible beneficial health effects of polyunsaturated fatty acids (PUFA) such as DHA and eicosapentaenoic acid (EPA) have led to major commercial developments of these conditionally essential polyunsaturated fatty acids for use in a variety of products for human consumption, animal feed, and cosmetics (*1, 2*). The stability of oils depends on various factors, but mainly on the fatty acid composition of the oil, the content of natural antioxidants, and the presence of oxygen as well as different storage and packaging conditions (*3*). Edible oils consist mainly of triacylglycerols (TAG, 95%); non-triacylglycerols (also known as minor components or unsaponifiable matter) make up the remaining 5%. These minor components are naturally occurring compounds with antioxidative properties that may contribute to the stability of oils against oxidation and quality deterioration (*4, 5*). The minor components of vegetable oils are primarily composed of phospholipids, tocopherols, tocotrienols, flavonoids such as flavones and other phenolic compounds, pigments (carotenoids, chlorophylls), sterols, and free fatty acids, as well as mono- and diacylglycerols (*4*). Several classes of these components might be present in each oil and contribute to its oxidative stability (*4*).

A number of chemical, instrumental, and sensory techniques are commonly used to monitor the oxidation of foods and to predict their shelf life and stability. These techniques can be used to evaluate the effectiveness of antioxidants in different lipid systems (*6*). Although sensory methods are most accurate in predicting the stability of lipids, they are cumbersome and do not always lend themselves to routine analysis (*7*).

Peroxide value (PV) and conjugated dienes (CD) are frequently used to measure primary oxidation products and lipid hydroperoxides. Meanwhile, thiobarbituric acid-reactive substances (TBARS) and headspace volatiles are employed as indicators for monitoring secondary products of oxidation such as aldehydes and other carbonyl compounds (*8*).

Little is known about the oxidative stability (OS) of algal oils, and virtually nothing is known about the OS of these oils when they have been stripped of their minor components. This research was designed to evaluate the OS of nonstripped and stripped algal oils such as ARASCO, DHASCO, and OMEGA-GOLD oils and to determine some of the minor components in nonstripped and stripped oils such as pigments (carotenoids and chlorophylls) and antioxidants (tocopherols

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and phenolics). Finally, the minor components of the oils were extracted into methanol and their free radical scavenging properties determined.

MATERIALS AND METHODS

Materials. Algal oils, namely, DHASCO containing 47.4% DHA and ARASCO containing 40−50% ARA, were obtained from Martek Bioscience Corp. (Columbia, MD). OMEGA-GOLD oil is the commercial name for an oil from single-cell microalgae, containing 41% DHA and 18% n-6 DPA, and was a product from Monsanto (St. Louis, MO); this oil is now being marketed by Martek Bioscience Corp. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin−Ciocalteu reagent, 2-thiobarbituric acid (2-TBA), gallic acid (3,4,5-trihydroxybenzoic acid), silicic acid powder (mesh size = 100−200, acid-wash), and tocopherol samples were obtained from Sigma Chemical Co. (St. Louis, MO). Activated charcoal was acquired from BDH Inc. (Toronto, ON, Canada). Compressed air, hydrogen, and ultrahigh-purity (UHP) helium were purchased from Canadian Liquid Air Ltd. (St. John's, NL, Canada). Hexane, methanol, sulfuric acid, isooctane, and 1-butanol were obtained from Fisher Scientific Co. (Nepean, ON, Canada).

Methods. *Preparation of Minor-Component-Stripped Oils.* ARASCO, DHASCO, and OMEGA-GOLD oils were stripped of their minor components, including free fatty acids, essentially according to the method of Khan and Shahidi (*9*) with minor modifications. A chromatographic column (3.4 cm i.d. × 40 cm height) was connected to an aspirator vacuum pump and packed sequentially with two adsorbents. The first layer in the column consisted of 50 g of activated silicic acid, the second layer was 50 g of activated charcoal, and the top layer was another 50 g of activated silicic acid. All adsorbents were suspended in *n*-hexane. The silicic acid (100 g), before introduction to the solvent, was activated as described by Min (*10*) by washing three times with a total of 3 L of distilled water. After each treatment, the silicic acid was left to settle for 30 min, and then the suspended silicic acid was decanted. Finally, the silicic acid was washed with methanol and the supernatant decanted. Traces of methanol were removed by filtration through a Büchner funnel under vacuum, and the semidried material was activated at 200 °C for 22 h.

Oil (50 g) was diluted with an equal volume of *n*-hexane and passed through the chromatographic column. The solvent in the eluent (stripped oil) was evaporated under vacuum at 30 °C, and traces of the solvent were removed by flushing with a stream of nitrogen. Stripped oils (stripped OMEGA-GOLD oil, SO; stripped DHASCO, SD; and stripped ARASCO, SA) were obtained and transferred into 10 mL bottles, flushed with nitrogen, and kept at −70 °C for subsequent studies.

Preparation of Samples for Schaal Oven and Photooxidation Tests. Stripped and nonstripped oil samples (0.5 g in 2 mL vials) were used to study their oxidative stability in the dark upon heating and under the light (photooxidation). For accelerated oxidation at 60 °C, the sample containers were placed in a forced-air oven (model 2, Precision Scientific Co., Chicago, IL), that is, under Shaal oven conditions (*9*). For photooxidation studies the samples were placed in a box (70 cm lengh × 35 cm width × 25 cm height) equipped with two 40 W cool white fluorescent lights, which were suspended approximately 10 cm above the surface of the oil containers. The inside and top of the box were covered with aluminum foil. The fluorescent radiation was at a level of 2650 lx, and the temperature inside the container was 27 ± 1 °C (*9*). Oil samples were removed from the oven after 1, 3, 5, and 7 days and from the light box after 4, 8, 12, and 24 h, cooled and flushed with nitrogen, covered with Parafilm, and kept at −70 °C for oxidative stability tests.

Oxidative Stability Tests. The oxidative stability of stripped and nonstripped oils was evaluated by determining conjugated dienes (CD), 2-thiobarbituric acid-reactive substances (TBARS), and headspace volatiles.

Determination of CD. CD of the oil samples were determined according to the IUPAC method (*11*). Oil samples (0.02−0.04 g) were weighed into 25 mL volumetric flasks, dissolved in isooctane (2,2,4-trimethylpentane), and made up to the mark with the same solvent. The contents were mixed thoroughly, and the absorbance was read at 234 nm in a 10 mm Hellma quartz cell using a Hewlett-Packard 8452A diode array spectrophotometer (Agilent, Palo Alto, CA). Pure isooctane was used as the blank. CD were calculated according to the equation

$$\mathrm{CD} = A/(c \times d)$$

where A = absorbance of the solution at 234 nm, C = concentration of the solution in g/100 mL of solution, and d = length of the cell in cm.

Determination of TBARS. Oil samples (0.05−0.20 g) were analyzed for their contents of TBARS according to the AOCS (*12*) method. The samples were accurately weighed into 25 mL volumetric flasks and made up to the mark with 1-butanol. This mixture (5 mL) was transferred into a dry test tube, and then 5 mL of fresh TBA reagent (200 mg of TBA in 100 mL of 1-butanol) was added to it. The contents were mixed and heated in a water bath at 95 °C for 120 min. The intensity of the resultant colored complex was measured at 532 nm using a Hewlett-Packard 8452A diode array spectrophotometer (Agilent). The TBARS values were calculated by multiplying the absorbance readings by a factor of 0.415 determined from a standard line prepared using 1,1,3,3-tetramethoxypropane as precursor of malonaldehyde (*9*, *13*).

Headspace Analysis of Volatiles. A Perkin-Elmer 8500 gas chromatograph equipped with an HS-6 headspace sampler (Perkin-Elmer Co., Montreal, QC, Canada) was used for volatile analysis of oil samples (*14*). The column used to separate the volatiles was a Supelcowax-10 fused-silica capillary (30 m × 0.32 mm i.d., 0.10 μm film; Supelco Canada Ltd., Mississauga, ON, Canada). Helium (UHP) was the carrier gas employed at an inlet column pressure of 20 psig with a split ratio of 7:1. The injector and flame ionization detector (FID) temperatures were 280 °C. The oven temperature was maintained at 40 °C for 5 min and then increased to 115 °C at 10 °C/min and subsequently ramped to 200 °C at 30 °C /min and held there for 5 min.

Oil samples (0.2 g) were transferred into special glass vials, which were capped with butyl septa, crimped, and analyzed. Vials were preheated in the HS-6 magazine assembly at 90 °C for a 45 min equilibrium period. Pressurization time was 6 s, and the volume of the vapor phase drawn was approximately 1.5 mL. Individual volatile compounds were tentatively identified by comparing the relative retention times of GC peaks with those of commercially available standards. Quantitative determination of dominant aldehydes, hexanal, and/or propanal was accomplished using 1% 2-heptanone (in stripped corn oil) as an internal standard. Formation of total volatiles was monitored as a measure of oxidation of the oil samples.

Preparation of Extracts from Oils. A measured amount of oil sample (20 g) was diluted with hexane (1:10, w/v) and extracted with methanol (10:2, v/v, hexane/methanol) three times at room temperature. The methanol extract was washed with hexane (1:1, v/v), and the methanol was completely removed under vacuum. The extract was redissolved in 10 mL of methanol, flushed with nitrogen, and kept at −70 °C for further analysis.

Evaluation of Phenolic Content and Antioxidative Activity of Methanolic Extracts from Oils. Determination of Total Phenolic Content. The total phenolic content was determined according to the procedure explained by Singleton and Rossi (*15*) with minor modifications. One milliliter of Folin−Ciocalteu reagent was added to 50 mL centrifuge tubes containing 1 mL of the extracts (0.2 g/mL) prepared previously. Contents were mixed thoroughly, and 1.5 mL of 20% sodium carbonate was added; the final volume was then made up to 10 mL with distilled water and mixed again. After 2 h of reaction at room temperature, the absorbance of the mixture was read at 765 nm; the value obtained was then used to calculate the phenolic contents using a standard curve prepared with gallic acid. Total extracted phenolics were expressed as milligrams of gallic acid equivalents, a common reference compound, per milliliter of extract.

DPPH Radical Scavenging Activity. One milliliter of freshly prepared DPPH radical solution (0.125 mM) was added to 1 mL of the prepared extract (0.2 g/mL) and mixed well to start the radical−antioxidant reaction. The absorbance at 517 nm was read against a blank of pure methanol after 0, 2, 4, 6, 8, 10, 15, and 20 min of reaction and used to estimate the remaining radical levels according to the standard curve. The reference antioxidant used was α-tocopherol. The inhibition

Table 1. Chemical Characteristics of Original Nonstripped and Stripped ARASCO, DHASCO, and OMEGA-GOLD Oils (Prior to Accelerated Oxidation)[a]

characteristic		N-ARASCO	S-ARASCO	N-DHASCO	S-DHASCO	N-OMEGA-GOLD	S-OMEGA-GOLD
oxidative status	conjugated dienes	3.87 ± 0.07b	1.67 ± 0.25a	10.27 ± 0.19ef	8.83 ± 0.02d	10.03 ± 0.11ef	6.10 ± 0.06c
	TBARS (μmol/g)	8.53 ± 0.06cb	5.27 ± 0.02a	13.88 ± 0.44e	11.44 ± 0.31d	14.05 ± 0.02f	8.01 ± 0.07bc
tocopherols (mg/kg)	α	100b	0	250c	0	30a	0
	γ	660b	0	810c	0	650a	0
	δ	30a	0	80c	0	50b	0
	total	790b	0	1140c	0	730a	0
pigments[b,c] (absorbance)	430 nm	0.11c	0.02b	0.7f	0.38e	0.15d	0a
	460 nm	0.06d	0.01ba	0.4c	0.05c	0.1e	0.01ab
	550 nm	0.01cde	0.01dce	0.01ecd	0ab	0.03f	0ba
	620 nm	0.01de	0.01ed	0abc	0bac	0.03f	0cab
	670 nm	0.01e	0a	0abcd	0abcd	0.03f	0abcd

[a] Values are means of three determinations ± standard deviations. For pigments, standard deviations were ≤0.01 and hence are not recorded. Values with different letters in each row are significantly different ($p < 0.05$) from one another. Abbreviations: N-ARASCO, nonstripped ARASCO; N-DHASCO, nonstripped DHASCO; N-OMEGA-GOLD, nonstripped OMEGA-GOLD oil; S-ARASCO, stripped ARASCO; S-DHASCO, stripped DHASCO; S-OMEGA-GOLD, stripped OMEGA-GOLD oil. [b] The ratio of oil to hexane (v/v) was 1:3 for ARASCO and OMEGA-GOLD and 1:6 for DHASCO. [c] Absorbance between 430 and 460 nm indicates the presence of carotenoids, and absorbance between 550 and 710 nm indicates the presence of chlorophylls.

Table 2. Fatty Acid Composition (Area Percent) of Nonstripped and Stripped ARASCO, DHASCO, and OMEGA-GOLD Oils[a]

fatty acid	N-ARASCO	S-ARASCO	N-DHASCO	S-DHASCO	N-OMEGA-GOLD	S-OMEGA-GOLD
C10:0			1.37 ± 0.18ab	1.24 ± 0.04ab		
C12:0			6.79 ± 0.76ab	6.44 ± 0.17ab		
C14:0			17.7 ± 0.91cd	17.4 ± 0.25cd	8.73 ± 0.39ab	9.08 ± 0.01ab
C16:0	8.29 ± 0.06ab	8.51 ± 0.03ab	12.1 ± 0.42cd	12.2 ± 0.05cd	23.4 ± 1.04e	32.0 ± 0.49f
C16:1			2.19 ± 0.07db	2.19 ± 0.03c	1.61 ± 0.07a	2.06 ± 0.02bd
C18:0	8.98 ± 0.03a	9.85 ± 0.06b				
C18:1n-9	18.0 ± 0.05c	20.1 ± 0.08d	15.9 ± 0.85ab	16.4 ± 0.31ab		
C18:2n-6	7.21 ± 0.03d	7.04 ± 0.03c	0.65 ± 0.00ab	0.65 ± 0.00ab		
C18:3n-6	2.87 ± 0.02b	2.47 ± 0.02a				
C20:3n-6	2.52 ± 0.02ab	2.52 ± 0ab				
C20:4n-6	42.8 ± 0.16d	40.2 ± 0.15c			2.45 ± 0.38ab	2.49 ± 0.04ab
C20:5n-3	1.94 ± 0.03b	2.34 ± 0.02c			3.21 ± 0.13d	1.28 ± 0.01a
C22:5n-6	1.76 ± 0.05ab	2.11 ± 0.03ab			13.3 ± 0.64c	17.2 ± 0.26d
C22:6n-3			41.0 ± 0.45cd	40.9 ± 0.12cd	34.5 ± 1.42b	25.9 ± 0.31a
others	5.63d	4.93c	2.31a	2.58b	12.84f	7.56e
total PUFA	59.1 ± 0.010f	56.6 ± 0.15e	41.7 ± 0.66ab	41.6 ± 0.33ab	53.4 ± 1.80d	46.4 ± 0.20c

[a] Values are mean values of triplicate determination ± standard deviation. Abbreviations: N-ARASCO, nonstripped ARASCO; N-DHASCO, nonstripped DHASCO; N-OMEGA-GOLD, nonstripped OMEGA-GOLD oil; S-ARASCO, stripped ARASCO; S-DHASCO, stripped DHASCO; S-OMEGA-GOLD, stripped OMEGA-GOLD oil.

percentage was calculated according to the method of Lee et al. (*16*) using the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample})/\text{absorbance of control}] \times 100$$

Analysis of the Oils. Analysis of Fatty Acid Composition. The fatty acid composition of the oils was determined according to the method described by Wanasundara and Shahidi (*17*). Fatty acid methyl esters (FAMEs), prepared as above, were analyzed using a Hewlett-Packard 5890 II gas chromatograpgh (Agilent) equipped with a 30 m × 0.25 mm, 0.25 μm film thickness, Supelcowax-10 column (SP 2330, Supelco Canada Ltd.). The injector and flame ionization detector temperatures were both at 270 °C. The oven temperature was initially 220 °C for 10 min and 15 s and then increased to 240 °C at 30 °C/min and held there for 9 min. Helium (UHP) was used as the carrier gas. The FAMEs were identified by comparing their retention times with those of an authentic standard mixture (GLC-461; Nu-Check). Results were recorded as weight percentages.

Measurement of Pigments. Pigments present in the stripped and nonstripped oil samples were determined qualitatively by measuring the absorbance at 430−460 nm for carotenoids and the absorbance at 550−710 for chlorophylls and their derivatives (*18*). The oil sample was mixed with hexane (1:6 for DHASCO and 1:3 for ARASCO and OMEGA-GOLD oils) and transferred into Hellma glass cells, and the absorbance was read using a Genesys 5 spectrophotometer (Spectronic Instruments, Inc., Rochester, NY) and by recording the absorption spectra between 430 and 710 nm.

Determination of Tocopherols by High-Performance Liquid Chromatography (HPLC). For γ- and δ-tocopherol analysis, a Shimadzu (Kyoto, Japan) HPLC system equipped with two LC-10AD pumps, an SPD-M10A diode array detector, and an SCL AA system controller was employed. The conditions of separation were as follows: a prepacked LUNA SILICA (2) column, Phenomenex (25 cm, 4.6 mm in diameter, 5 μm particles) (Aschaffenburg, Germany); mobile phase, 4% dioxane in hexane; flow rate, 1.5 mL/min; injection volume, 20 μL; and detector setting, 295 nm. A Shimadzu HPLC system (Kyoto, Japan) was used for α-tocopherol analysis (LC 10AD pumps, RF-535 fluorescence detector, C-R4A Chromatopac). The conditions of separation were as follows: prepacked LUNA SILICA (2) column, Phenomenex (25 cm, 4.6 mm in diameter, 5 μm particles) (Aschaffenburg, Germany); mobile phase, 0.5% isopropanol in hexane; flow rate, 1 mL/min; injection volume, 20 μL; and detector settings, Ex 290 nm and Em 330 nm. One gram of stripped and nonstripped oil sample was dissolved in 10 mL of mobile phase, passed through a 0.45 μm filter, and injected onto the HPLC column (Hoffman-La Roche Ltd., Basel, Switzerland).

Statistical Analysis. All experiments were performed in triplicate and the results reported as mean ± standard deviation. Normality was examined using Sigmastat. Analysis of variance and Tukey's standardized test were performed at a level of $p < 0.05$ to assess the significance of differences among mean values.

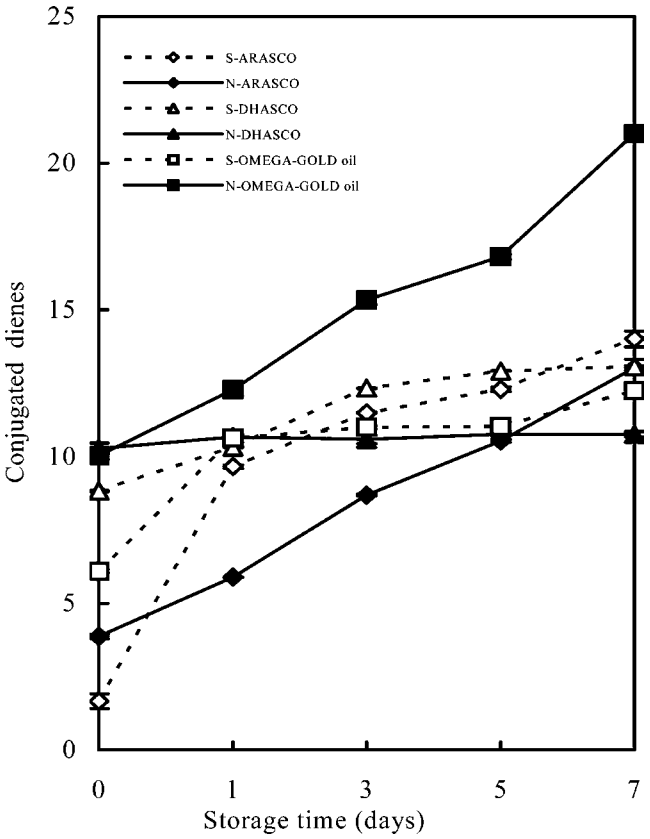

Figure 1. Conjugated dienes of nonstripped and stripped ARASCO, DHASCO, and OMEGA-GOLD oils stored under Schaal oven conditions at 60 °C.

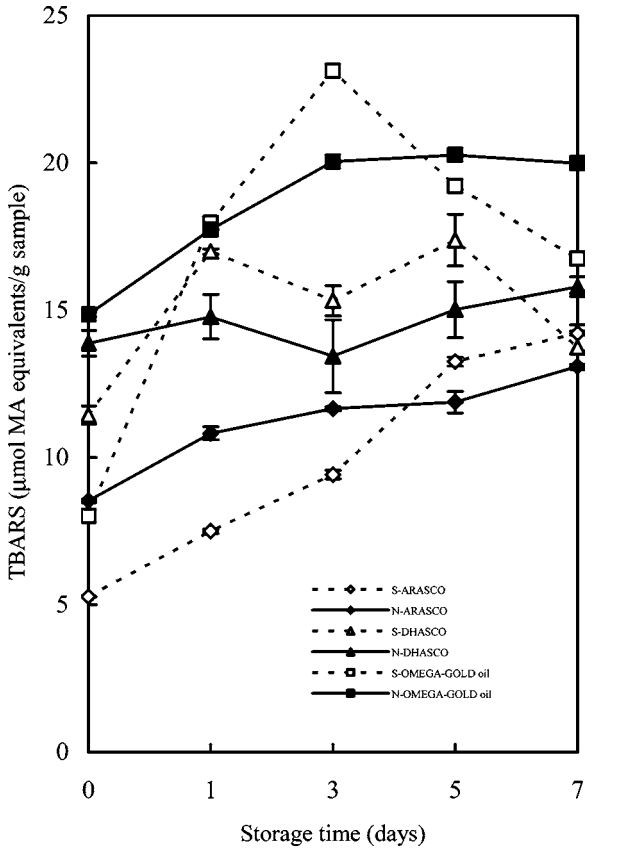

Figure 2. TBARS of nonstripped and stripped ARASCO, DHASCO, and OMEGA-GOLD oils stored under Schaal oven conditions at 60 °C.

RESULTS AND DISCUSSION

Analysis of Nonstripped and Stripped Oil Samples. *Chemical Characteristics of Nonstripped and Stripped ARASCO, DHASCO, and OMEGA-GOLD Oils.* **Table 1** summarizes the chemical characteristics of nonstripped and stripped ARASCO, DHASCO), and OMEGA-GOLD oils. The original samples of nonstripped ARASCO (N-ARASCO), nonstripped DHASCO (N-DHASCO), and nonstripped OMEGA-GOLD oils (N-OMEGA-GOLD oil) contained higher ($p < 0.05$) amounts of primary (CD) and secondary (TBARS, propanal and/or hexanal, as well as total volatiles) oxidation products than their corresponding stripped ARASCO (S-ARASCO), stripped DHASCO (S-DHASCO), and stripped OMEGA-GOLD oils (S-OMEGA-GOLD). Similar results were obtained by Khan and Shahidi (*9*) for borage and evening primrose oils, and this was explained to be due to the removal of oxidation products upon stripping; the resultant stripped oils had good oxidative status compared to their nonstripped counterparts.

The main tocopherols, as determined by HPLC, are summarized in **Table 1**. NDHASCO had the highest ($p < 0.05$) total tocopherols compared to N-ARASCO and N-OMEGA-GOLD oils; high amounts of tocopherols might contribute to the superior stability of DHASCO. Two hundred and fifty parts per million of α-tocopherol, 810 ppm of γ-tocopherol, and 80 ppm of δ-tocopherol were detected in N-DHASCO; corresponding amounts in ARASCO were 100, 660, and 30 ppm, respectively. Meanwhile, OMEGA-GOLD oil, similar to DHASCO and ARASCO, following stripping contained no tocopherols, thus confirming that the stripping procedure was effective in the removal of tocopherols from all oil samples examined.

Pigments such as carotenoids, with absorbance between 430 and 460 nm (*18*), were detected. The content of carotenoids in N-DHASCO was significantly ($p < 0.05$) higher than those present in N-ARASCO and N-OMEGA-GOLD oil (**Table 1**). However, chlorophylls existed in higher levels in N-OMEGA-GOLD oil than in N-ARASCO, and no chlorophylls were present in N-DHASCO. By comparing pigments found in nonstripped oils and their counterparts, it is clear that stripped oils had much less ($p < 0.05$) pigments than their nonstripped counterparts (**Table 1**) as indicated by the absorbance of pigments at different wavelengths. For example, the absorbance at 430 nm for N-ARASCO was 0.11, which was higher $p < 0.05$ than that of S-ARASCO (0.02). Similarly, the absorbance at 430 nm for N-DHASCO was 0.7, which was also higher ($p < 0.05$) than that of S-DHASCO (0.37). Meanwhile, stripping OMEGA-GOLD oil of its carotenoids was more effective compared to the stripping of the other two algal oils (ARASCO and DHASCO) as indicated by no detectable absorbance for S-OMEGA-GOLD oil compared to 0.15 for N-OMEGA-GOLD oil at 430 nm.

Algal oils were stripped of their minor components using a modified multilayer column chromatographic technique developed by Lampi et al. (*19*). This procedure required only 2 h to strip 50 g of algal oil and was more effective for stripping OMEGA-GOLD oil compared to ARASCO, but it was not effective enough in stripping DHASCO of all of its pigments. It is clear that the chlorophylls were more easily removed from the oils examined by silicic acid and charcoal than carotenoids. This might be related to the existing differences in the chemical structures of chlorophylls and carotenoids that enable chlorophylls to be adsorbed by the stationary phase to a greater extent than carotenoids.

Fatty Acid Composition of Nonstripped and Stripped ARASCO, DHASCO, and OMEGA-GOLD Oils. **Table 2** illustrates the fatty acid composition of N-ARASCO, N-DHASCO, and N-OMEGA-GOLD oils and their stripped counterparts,

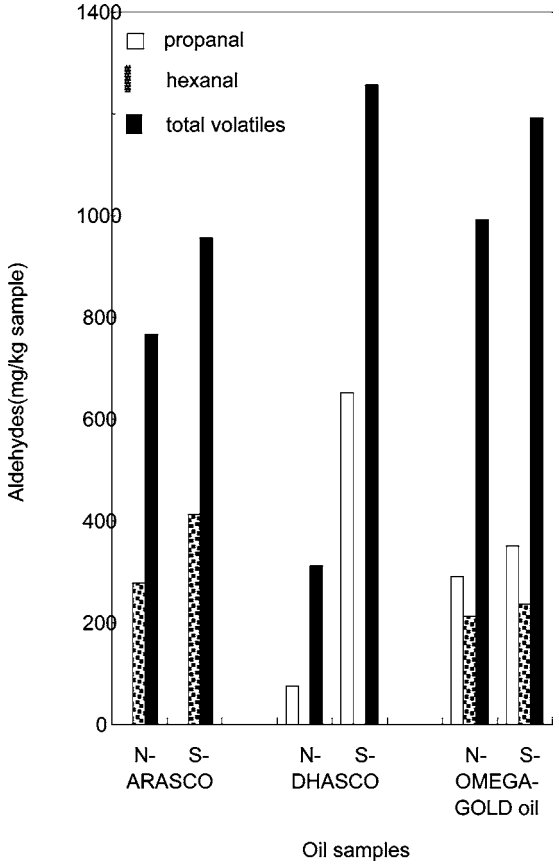

Figure 3. Propanal, hexanal, and total volatiles of nonstripped and stripped ARASCO, DHASCO, and OMEGA-GOLD oils stored under Schaal oven conditions at 60 °C.

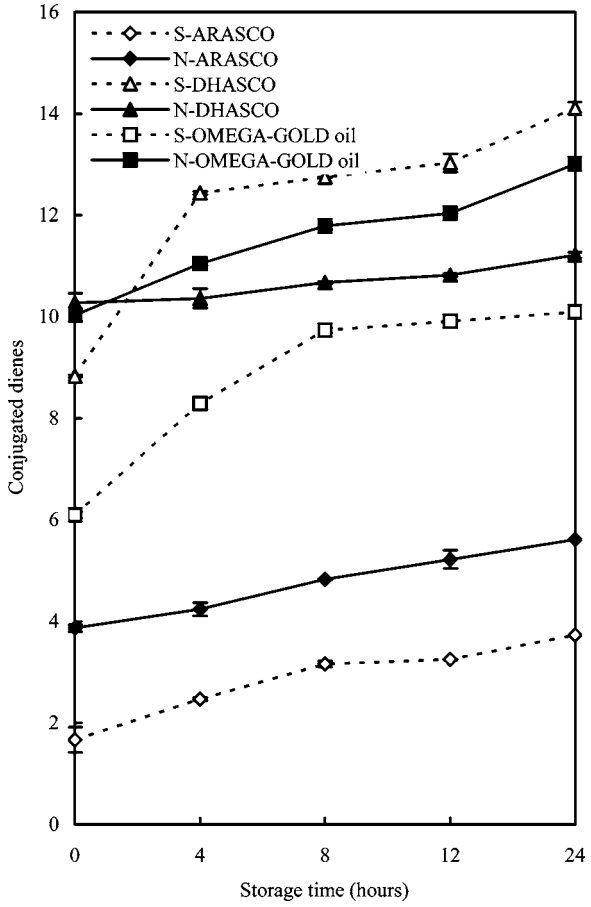

Figure 4. Conjugated dienes of nonstripped and stripped ARASCO, DHASCO, and OMEGA-GOLD oils stored under fluorescent light at 27 °C.

S-ARASCO, S-DHASCO, and S-OMEGA-GOLD oils. The results in this table indicate that nonstripped and stripped ARASCO contained significantly higher amounts ($p < 0.05$) of total PUFA than nonstripped and stripped DHASCO and OMEGA-GOLD oils. The results of fatty acid analysis of oils examined were similar to those reported by Hamam and Shahidi (*20*−*22*). The dramatic change in the fatty acid profile of OMEGA-GOLD oil following stripping cannot be explained, and further work in this area is in progress.

Oxidative Stability of Nonstripped and Stripped ARASCO, DHASCO, and OMEGA-GOLD Oils Stored under Schaal Oven Conditions at 60 °C. *Primary Oxidation Products.* **Figure 1** indicates that N-DHASCO was more ($p < 0.05$) stable than its corresponding stripped counterpart (S-DHASCO). Meanwhile, N-ARASCO and N-OMEGA-GOLD oils and their counterparts were unstable under these conditions. The stability of N-DHASCO under Schaal oven conditions might be due to the higher amount of tocopherols present in N-DHASCO compared to the amounts detected in N-ARASCO and N-OMEGA-GOLD oils (**Table 1**). However, S-ARASCO, S-DHASCO, and S-OMEGA-GOLD oils were devoid of these minor components, which makes them unstable under Schaal oven conditions (forced-air oven at 60 °C) (*21*).

Secondary Oxidation Products. Secondary oxidation products of nonstripped and stripped algal oils were determined by examining TBARS and headspace volatiles, mainly hexanal and propanal. When N-ARASCO, N-DHASCO, and N-OMEGA-GOLD oils were examined under Schaal conditions, the TBARS values were lower than those of their corresponding stripped counterparts (**Figure 2**). However, the change in TBARS values for N-ARASCO and N-OMEGA-GOLD oils were higher ($p < 0.05$) than those of N-DHASCO, which indicates the relative instability of N-ARASCO and N-OMEGA-GOLD oils. This might be due to the presence of high amounts of minor components, including tocopherols, in DHASCO. The results reported here agree with those of Hamam and Shahidi (*20*), who found that TBARS values for DHASCO remained constant during the storage period, indicating good stability of this oil under Schaal oven conditions, whereas ARASCO (*20*) and OMEGA-GOLD oils (*22*) were less stable under the same conditions.

The major volatile detected during the oxidation of ARASCO (omega-6 algal oil) was hexanal (**Figure 3**), whereas propanal was the major volatile produced during the oxidation of DHASCO (**Figure 3**); both hexanal and propanal were detected during the oxidation of OMEGA-GOLD oil (**Figure 3**). N-DHASCO was more stable ($p < 0.05$) than its stripped counterpart as indicated by higher ($p < 0.05$) propanal content in stripped DHASCO than in N-DHASCO (**Figure 3**). Meanwhile, nonstripped and stripped ARASCO and OMEGA-GOLD oils were not stable under Schaal oven conditions. Hexanal content in both stripped and nonstripped ARASCO was high (**Figure 3**), whereas propanal and hexanal contents were also high in both stripped and nonstripped OMEGA-GOLD oils (**Figure 3**).

Oxidative Stability of Nonstripped and Stripped ARASCO, DHASCO, and OMEGA-GOLD Oils under Fluorescent Light at 27 °C. *Primary Oxidation Products.* Formation of conjugated dienes occurred in stripped ARASCO, DHASCO, and OMEGA-GOLD oils exposed to fluorescent light as demonstrated in **Figure 4**. This might also arise from pigments (mainly carotenoids) that were retained in the stripped oils

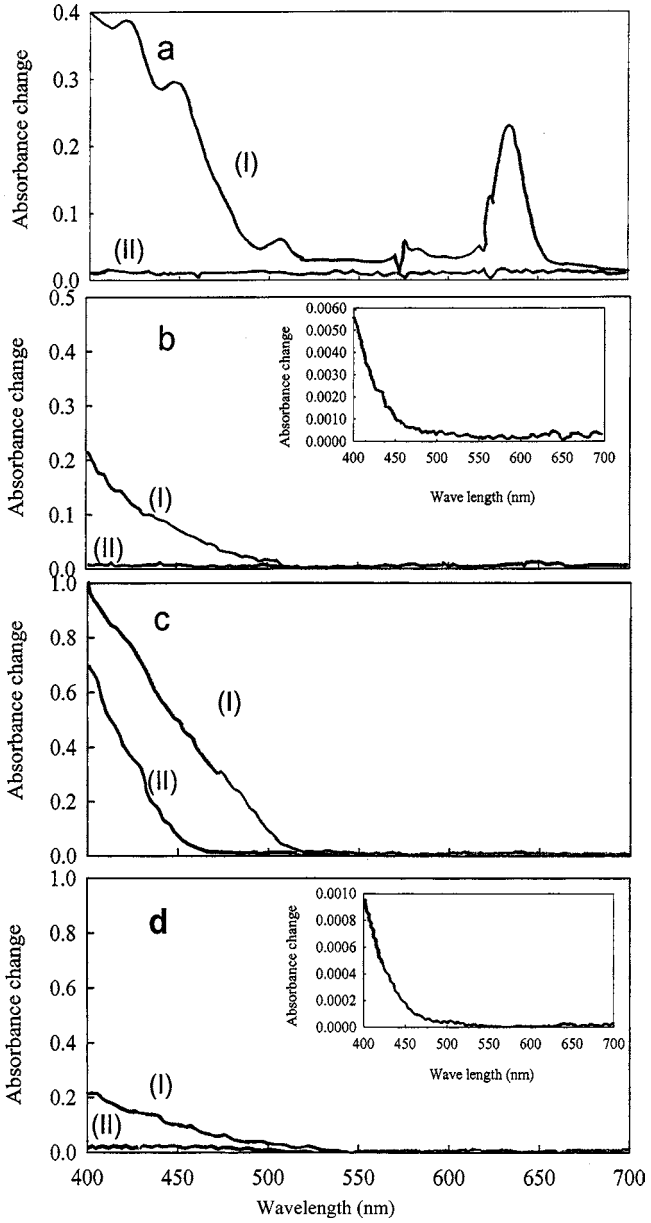

Figure 5. Visible spectra of pigments in oil/hexane of nonstripped (I) and stripped (II) olive (**a**), ARASCO (**b**), DHASCO (**c**), and OMEGA-GOLD oils (**d**). Oil/hexane ratios were 1:1 (v/v) for olive oil, 1:3 (v/v) for ARASCO and OMEGA-GOLD oils, and 1:6 (v/v) for DHASCO. Spectra II are magnified in the upper right corners of panels **b** and **d**.

(**Figure 5**). Similarly, an increase in CD values was observed for nonstripped ARASCO and OMEGA-GOLD oils. However, N-DHASCO was stable under light.

Secondary Oxidation Products. TBARS values of nonstripped and stripped ARASCO, DHASCO, and OMEGA-GOLD oils under fluorescent light are illustrated in **Figure 6**. Data in this figure indicate that N-DHASCO had the highest stability compared with other oils as the TBARS values changed minimally. Meanwhile, N-ARASCO and N-OMEGA-GOLD oils were unstable under the same conditions. However, S-ARASCO, S-DHASCO, and S-OMEGA-GOLD oils were less stable than their nonstripped counterparts under fluorescent light. This might be due to the retention of some of the pigments, mainly carotenoids, in S-ARASCO, S-DHASCO, and S-OMEGA-GOLD oils (**Figure 5**), which might contribute to the instability of the stripped oils. Carotenoids are known to act both as antioxidants by scavenging singlet oxygen and as

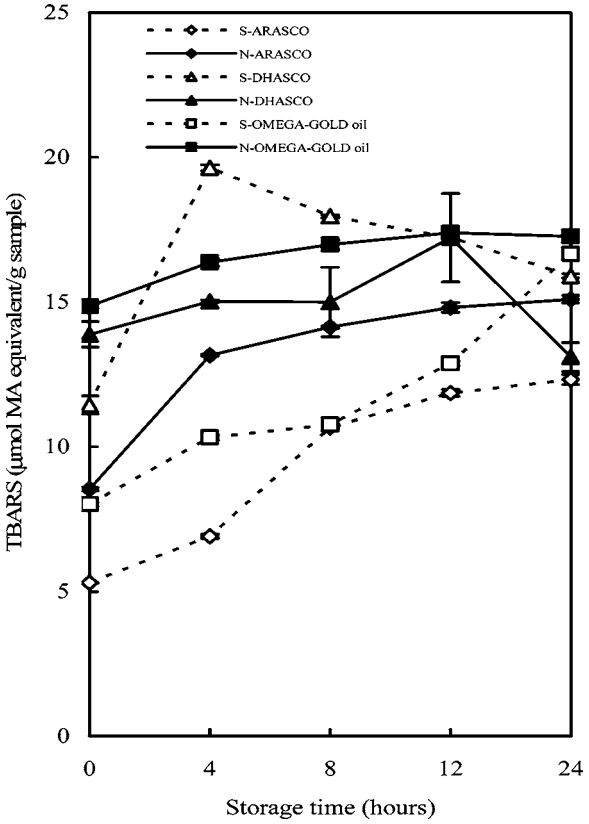

Figure 6. TBARS of nonstripped and stripped ARASCO, DHASCO, and OMEGA-GOLD oils stored under fluorescent light at 27 °C.

pro-oxidant under certain conditions (*23*). The latter mechanism seems to be operative under experimental conditions employed in this work. These effects generally are quite important and may overwhelm the system despite the presence of certain other compounds. S-DHASCO, devoid of most of its antioxidants, but with retention of some caroteniods, may oxidize rapidly as indicated by the formation of high amounts of TBARS. N-DHASCO was more stable than its stripped counterpart on the basis of propanal contents (**Figure 7**), possibly due to partial retention of carotenoid pigments. Therefore, removal of antioxidants, but not all of the carotenoids, was responsible for this observation. However, both nonstripped and stripped ARASCO and OMEGA-GOLD oils were unstable under fluorescent light, as indicated in the total volatile content found in S-ARASCO and OMEGA-GOLD oils (**Figure 7**).

Radical Scavenging Activity of ARASCO, DHASCO, and OMEGA-GOLD Oils. Examination of the free radical scavenging properties of selected oils might identify their potential utilization in promoting human health. Generally known antioxidants, including radical scavengers, might protect important biomolecules from radical attacks and consequently reduce the risk of aging-associated health problems, such as cancer and heart disease (*24*).

Determination of Total Phenolic Contents of Oil Samples. It is well-known that phenolic compounds, including tocopherols, contribute to the overall antioxidant capacity of oils. Phenolics have a great influence on the stability, sensory, and nutritional characteristics of oil samples and might prevent their deterioration through quenching of radical reactions responsible for lipid oxidation (*25*, *26*). It has been reported that oil stability is correlated not only with the total amount of phenolics but also with the type of phenolics present (*26*). The total phenolics of algal oils, obtained by methanol extraction, are given as gallic acid equivalents (GAE). The total phenolics are also expressed

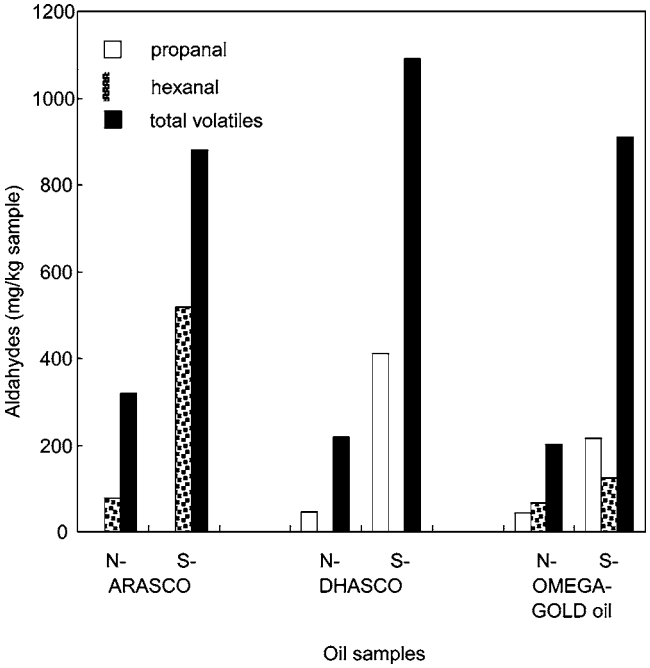

Figure 7. Propanal, hexanal, and total volatile contents in nonstripped and stripped ARASCO, DHASCO, and OMEGA-GOLD oils under fluorescent light at 27 °C.

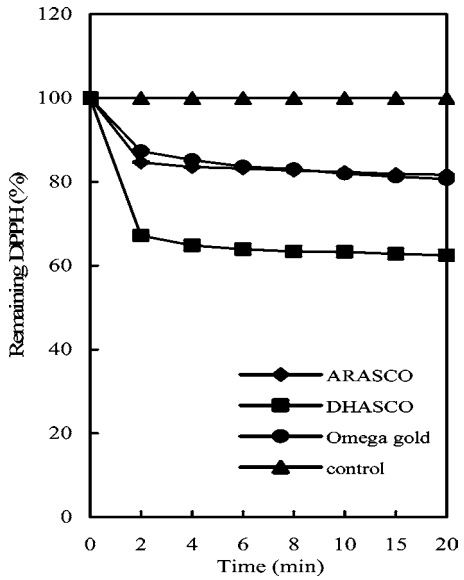

Figure 8. Reaction of ARASCO, DHASCO, and OMEGA-GOLD oil extracts with DPPH radical. The control indicates 100% DPPH radical remaining.

as GAE for ARASCO, DHASCO, and OMEGA-GOLD oil extracts. Data (not shown) demonstrate that DHASCO extract had the best ability ($p < 0.05$) to reduce the Folin−Ciocalteu reagent compared to ARASCO and OMEGA-GOLD oil extracts. These results support the high stability of DHASCO under Schaal oven conditions as shown earlier.

DPPH Radical Scavenging Activity of Algal Oil Methanolic Extracts. DPPH radical was used to evaluate free radical scavenging properties of algal oil extracts, mainly their minor components (**Figure 8**). Results shown in **Figure 8** demonstrate that the DHASCO extract exhibited the best ability to scavenge DPPH radical compared to ARASCO and OMEGA-GOLD oil extracts. Data indicated that DHASCO extract had the highest ($p < 0.05$) antioxidant capacity, expressed as α-tocopherol equivalents, compared to ARASCO and OMEGA-GOLD oil extracts, thus explaining the better stability of DHASCO compared to the other two algal oils tested.

Summary and Conclusions. Minor components of algal oils play a major role in their oxidative stability in the dark as well as light. However, the fatty acid composition of the oil and the total tocopherols as well as the type of pigments present contribute to their stability. The presence of these constituents reflects their food and supplement value as well as shelf life.

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