Oxidative Stability of Structured Lipids Produced from Borage (*Borago officinalis* L.) and Evening Primrose (*Oenothera biennis* L.) Oils with Docosahexaenoic Acid

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ABSTRACT: This study utilized γ-linolenic acid (18:3n-6; GLA)-rich borage oil (BO) and evening primrose oil (EPO) for the synthesis of structured lipids (SL) and compared the oxidative stability of the products with those of unmodified BO and EPO as controls. Immobilized Novozym 435 lipase from *Candida antarctica* was used as the biocatalyst for SL production. BO or EPO was enzymatically modified with docosahexaenoic acid (22:6n-3; DHA), as the acyl donor, to produce SL. The SL were characterized and their oxidative stabilities evaluated. Among the oils examined, SL gave rise to higher quantities ($P \leq 0.05$) of conjugated dienes, TBARS, and headspace volatiles as compared to their unmodified counterparts. Results indicated that modified oils were less stable than their unmodified counterparts. The double bond index (DBI) and methylene bridge index (MBI) of oils decreased ($P < 0.05$) during oxidation in the more unsaturated oils. An attempt was made to correlate various parameters of oxidation with DBI and MBI of oils; correlation coefficients ($-r$) were within the range of 0.574–0.973. This suggests that indicators such as DBI and MBI can reflect oxidative stability of oils.

Paper no. J10258 in *JAOCS 79*, 1003–1013 (October 2002).

KEY WORDS: Borage oil, *Candida antarctica* lipase, evening primrose oil, oxidative stability, structured lipids.

Structured lipids (SL) are TAG that have been modified to contain short- and/or medium- and long-chain fatty acyl moieties attached to a single glycerol molecule and that may provide a specific functionality (1). These specialty lipids may provide an effective means of delivering desired FA with particular characteristics to target specific disease conditions. SL may be synthesized chemically or *via* enzyme-catalyzed reactions. The products from enzymatic reactions are likely to be more desirable than those produced from chemical-assisted reactions (2). Furthermore, enzymatic processes, using stereospecific lipases, are employed in order to achieve products with desired structural configurations.

PUFA may be used in the preparation of SL. The n-3 PUFA, mainly eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), have been recognized for their important role in health. Research suggests that n-3 PUFA may reduce the risk of cardiovascular disease by lowering serum TAG, reducing the occurrence of arrhythmia, and acting as antiatherogenic and antithrombotic agents (3,4). DHA is now recognized as a physiologically essential nutrient in the brain and retina for neuronal functioning and visual acuity, respectively (5). On the other hand, the n-6 PUFA are required for certain physiological functions in the human body. γ-Linolenic acid (GLA; 18:3n-6), a pre-essential (conditionally essential) n-6 FA, is an important intermediate in the bioconversion of linoleic acid (LA; 18:2n-6) to eicosanoid precursor arachidonic acid (20:4n-6).

Borage and evening primrose oils are important sources of GLA; 17–23% of FA in borage (6) and 7–10% of those in evening primrose (7) oils consist of GLA. These specialty oils are frequently used in clinical and nutritional applications where it has been shown that diseases are possibly associated with an impaired Δ6 desaturase activity (7). This impairment may be alleviated by dietary supplementation of GLA, which is biosynthesized from LA by Δ6 desaturase. Evening primrose oil has been reported to be effective for curing rheumatoid arthritis (8) and multiple sclerosis (9). Dietary supplementation with evening primrose oil has been shown to produce a wide range of metabolites, including prostaglandin E_1 and 15-OH-dihomo-γ-linolenic acid, which have desirable effects on many organs and conditions, notably the cardiovascular system and inflammation (7). Furthermore, evening primrose oil has been found to inhibit the growth of mammary tumors in rats (10).

Lipid oxidation is one of the major causes of quality deterioration in lipid-containing foods. Despite their health benefits, borage and evening primrose oils containing PUFA are highly susceptible to rapid oxidative deterioration and thus experience problems with storage stability (2). It is important to prevent oxidation of edible oils and of foods that contain them in order to maintain their quality and safety. Oxidation of oils may be initiated by light, heat, and in the presence of metal ions. Oil oxidation occurs *via* a free-radical chain reaction mechanism involving initiation, propagation, and termination steps (11,12). Oxidative deterioration of edible oils involves autoxidation accompanied by both oxidative and nonoxidative reactions (13). The oxidation products from oils, which include hydroperoxides, decompose to produce a variety of volatile compounds that result in off-flavor and off-odor in oils.

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Studies on the synthesis of SL by incorporating n-3 FA (especially, EPA and DHA) into borage and evening primrose oils have previously been reported (1,14,15). In this paper, we describe the characteristics and oxidative stability of SL produced from borage and evening primrose oils with DHA.

MATERIALS AND METHODS

Materials. Borage oil was obtained from Bioriginal Food and Science Corporation (Saskatoon, Saskatchewan, Canada), and evening primrose oil was provided by Efamol, Inc. (Kentville, Nova Scotia, Canada). Algal oil (DHASCO®) containing DHA (47.4%) was obtained from Martek Biosciences Corporation (Columbia, MD). Immobilized lipase Novozym 435 from *Candida antarctica* was supplied by Novo Nordisk Biochem North America Inc. (Franklinton, NC). All other chemicals were American Chemical Society (ACS) grade or better.

Purification of DHA from algal oil. DHA concentrate (97.1% pure) was obtained from hydrolyzed algal oil using the urea–FA complexation method as described by Senanayake and Shahidi (16).

SL synthesis. SL of borage and evening primrose oils containing DHA were synthesized *via* acidolysis reaction using lipase from *C. antarctica* as the biocatalyst. Borage oil (300 mg) or evening primrose oil (297 mg) was mixed with DHA (120 mg) in screw-capped test tubes, and then Novozym 435 lipase (278–299 enzyme activity units) and water (2% by weight of substrates plus enzyme) were added; hexane (3 mL) was used as the solvent. The mixture was stirred in an orbital shaker at 250 rpm and 42–43°C. Individual sample vials were removed and analyzed at 24–26 h. The same procedures were employed for the controls, which were devoid of the enzyme.

Recovery of SL. The enzymes were removed by passing the reaction mixture through a bed of anhydrous sodium sulfate. Samples were placed in 250-mL conical flasks, and 20 mL of a mixture of acetone/ethanol (1:1, vol/vol) was added. The reaction mixture was titrated with 0.5 N NaOH to a phenolphthalein endpoint. The mixture was transferred to a separatory funnel and thoroughly mixed with 25 mL hexane. The lower aqueous layer was separated and discarded. The upper hexane layer containing acylglycerols was passed through a bed of anhydrous sodium sulfate. The acylglycerol fraction was subsequently recovered following hexane removal at 45°C using a rotary evaporator.

FA composition. The FA composition of modified (structured lipids) and unmodified oils was determined according to Senanayake and Shahidi (16) by means of GC.

Oil analyses prior to storage at 60°C. After preparation of enzymatically modified oils, their chemical properties, mainly PV, TBARS, iodine value, saponification value, and acid value, were determined using standard methodologies described by the American Oil Chemists' Society (17). Conjugated diene (CD) values of oil samples were measured by the method of IUPAC (18).

Oxidative stability experiments. Oxidative stability of SL as well as unmodified oils was carried out under Schaal-oven test conditions at 60°C. Each oil (1.8 g), in triplicate, was placed in test tubes (10 mm diameter and 12 cm height) and stored in a forced-air oven (Thelco, Model 2, Precision Scientific Co., Chicago, IL) at 60°C. Several different methods such as CD determination (18), TBARS value (17), and headspace volatiles analysis were chosen to follow the progress of lipid oxidation in the oils. The samples were removed periodically at 0, 6, 12, 24, 48, 72, and 96 h from the oven, cooled to room temperature, flushed with nitrogen for 30 s, capped, and stored at −80°C until analyzed.

Analysis of volatile oxidation products. A PerkinElmer 8500 gas chromatograph and an HS-6 headspace sampler (PerkinElmer Corp., Montréal, Canada) were used for the analysis of volatiles produced during storage of oil samples. The volatiles in the headspace of oxidized oils (obtained from the accelerated oxidation method) were separated using a high-polarity SUPELCOWAX 10 fused-silica capillary column (0.32 mm i.d., 30 m length, 0.10 μm film thickness; Supelco Canada Ltd., Oakville, Ontario, Canada). Ultra high purity helium was the carrier gas employed at an inlet column pressure of 17.5 psig with a split ratio of 7:1. The oven temperature was maintained at 40°C for 5 min and then ramped to 200°C at 20°C/min and held there for 5 min. The injector and FID temperatures were adjusted to 280°C and held at this temperature throughout the analysis.

For headspace analysis, 0.20 g of each oil was transferred to a 5-mL headspace vial (Chromatographic Specialties Inc., Brockville, Ontario, Canada). The vials were capped with Teflon-lined septa, crimped, and then frozen and kept at –20°C for further analyses. Analysis of oil was carried out within 7 d. The oxidative stability of the stored samples was monitored at the beginning and at the end of the storage period using CD value as an indicator. The CD values were normally less than 0.09. To avoid heat shock after removal of sample vials from storage, frozen vials were tempered at room temperature for 10 min and then preheated in the HS-6 magazine assembly at 90°C for a 40-min equilibration period. Pressurization time of the vial was 6 s, and the volume of the vapor phase drawn was approximately 1.5 mL. Chromatographic peak areas were expressed as integrator count units. Individual volatile compounds were tentatively identified by comparison of their retention times with those of commercially available standards. Quantitative determination of dominant volatiles (mainly hexanal and propanal) was accomplished using 2-heptanone as an internal standard.

Analysis of tocopherols. The content of tocopherols in oils was determined by HPLC by the method of Thompson and Hatina (19). Tocopherols in the oils were separated using a Lichrosorb Si-60 (3.2 × 20 mm, 5 μm; Merck, Darmstadt, Germany) analytical column by employing diethyl ether/hexane (5:95, vol/vol) as the mobile phase. The apparatus used for the analysis was a Shimadzu HPLC equipped with two LC-6A pumps, SPD-6AV UV-vis spectrophotometric detector, and C-R4A Chromatopac for data handling. A 20-μL sample (1 g of oil was dissolved in 10 mL of mobile

phase) and a flow rate of 1 mL/min were used for the analysis. The tocopherols in the oils were detected using a UV detector set at 295 nm and identified by comparing their retention times with those of known tocopherol standards.

Determination of double bond index (DBI). For determination of DBI, the number of double bonds contained in each FA was multiplied by its respective mole percentage and summed for all FA detected. The mean number of double bonds per fatty acyl chain was then expressed as the DBI (20).

Determination of methylene bridge index (MBI). The mean number of bis-allylic methylene bridge positions of FA constituents, expressed as methylene bridge index (MBI), was calculated by multiplying the number of bis-allylic methylene bridge positions contained in each FA species by its respective mole percentage and summed for all FA present (21).

Statistical evaluation. ANOVA and Tukey's Studentized test (22) were carried out based on data collected from triplicate determinations. Significance levels were established at $P \leq 0.05$ and $P \leq 0.005$.

RESULTS AND DISCUSSION

The FA composition of modified borage and evening primrose oils and their unmodified counterparts is given in Table 1. Modified borage and evening primrose oils contained 35.4 and 33.2% DHA, respectively. After enzyme-catalyzed acidolysis, the GLA content of borage and evening primrose oils was decreased from 23.5 to 16.1% and from 9.1 to 7.6%, respectively. DHA incorporation into oils resulted in a significant reduction in the content of (18:2n-6); LA was decreased from 37.8 to 25.2% in borage oil and from 72.6 to 48.5% in evening primrose oil. In both oils, changes were also observed in saturated and monounsaturated FA. Saturated FA 16:0 was decreased from 9.6 to 5.3% in borage oil and from 6.2 to 3.5% in evening primrose oil. The content of monounsaturated FA, such as 18:1, was reduced from 15.5 to 11.3% in borage oil and from 8.7 to 4.5% in evening primrose oil. The content of total tocopherols in both oils decreased during the enzyme-catalyzed acidolysis process (from 1,976 to 1,488 ppm in borage oil and from 1,120 to 730 ppm in evening primrose oil).

The chemical properties of unmodified borage and evening primrose oils and their enzymatically modified counterparts are reported in Table 2. The native oils had PV of 1.7–2.2 meq oxygen/kg compared to 1.9–2.7 meq/kg for their enzymatically modified counterparts (Table 2). The CD and TBARS values of enzymatically modified oils were similar to corresponding values of unmodified oils. The CD values of unmodified and modified oils were 1.9–2.1 and 2.0–2.4, respectively, whereas corresponding TBARS values were 1.2–1.4 and 1.1–1.6 μmol malonaldehyde equivalents/g of oil (Table 2). The iodine values of enzymatically modified oils were higher than those of their unmodified counterparts owing to a higher degree of unsaturation attained in the modified oils following incorporation of DHA. The iodine values of unmodified and modified oils were 141.7–153.9 and 198.4–207.7 g iodine/100 g oil, respectively (Table 2). However, the saponification values of enzymatically modified oils were lower than those of their unmodified counterparts. The unmodified and modified oils had saponification values of 269.4–273.5 and 118.7–142.5 mg KOH/g, respectively (Table 2). On the other hand, the acid values of modified oils were reduced to 0.04–0.05%. The acylglycerol composition of modified oils revealed that the relative content of TAG (88.5–90.2%) was much higher than that of DAG (8.8–9.0%) and MAG (0.8–2.6%).

The CD value of lipids, as reflected in absorption readings at 234 nm, is an indicator of oxidation as the non-CD arrangement of unsaturated lipids is changed to CD. Figure 1 shows the CD content of both modified and unmodified oils. When oxidation was induced at 60°C, the enzymatically modified oils had a higher measure of conjugated double bonds than

TABLE 1
FA Profile of Borage and Evening Primrose Oil-Based Structured Lipids[a]

	Borage oil		Evening primrose oil	
FA (wt%)	Unmodified	DHA-enriched	Unmodified	DHA-enriched
14:0	0.07 ± 0.02	0.04 ± 0.02	0.04 ± 0.01	0.03 ± 0.02
16:0	9.50 ± 0.50	5.33 ± 0.04	6.17 ± 0.09	3.52 ± 0.41
16:1	0.20 ± 0.05	0.20 ± 0.02	0.05 ± 0.02	0.06 ± 0.02
17:0	0.10 ± 0.01	ND	0.08 ± 0.01	ND
18:0	3.50 ± 0.03	2.10 ± 0.20	1.75 ± 0.12	1.31 ± 0.55
18:1	15.5 ± 0.70	11.3 ± 0.52	8.65 ± 0.56	4.53 ± 0.25
18:2n-6	37.8 ± 1.10	25.2 ± 1.20	72.6 ± 0.91	48.5 ± 1.18
18:3n-6	23.5 ± 0.85	16.1 ± 0.97	9.12 ± 0.38	7.60 ± 0.57
18:3n-3	0.21 ± 0.05	0.20 ± 0.05	0.16 ± 0.03	0.11 ± 0.02
20:0	0.22 ± 0.08	ND	0.34 ± 0.05	ND
20:1	4.20 ± 0.10	2.52 ± 0.20	0.29 ± 0.07	0.21 ± 0.11
20:2	0.21 ± 0.05	0.13 ± 0.07	0.05 ± 0.05	ND
22:0	0.15 ± 0.07	0.12 ± 0.04	ND	ND
22:1	2.35 ± 0.12	1.24 ± 0.10	0.14 ± 0.05	0.13 ± 0.05
22:6n-3	ND	35.4 ± 1.24	0.12 ± 0.01	33.2 ± 0.65

[a]Mean ± SD of triplicate determinations from different experiments. ND, not detected.

TABLE 2
Chemical Properties of Borage and Evening Primrose Oil-Based Structured Lipids[a]

Characteristic	Borage oil		Evening primrose oil	
	Unmodified	DHA-enriched	Unmodified	DHA-enriched
PV (meq/kg)	1.7 ± 0.4[a]	1.9 ± 0.9[a]	2.2 ± 0.5[a]	2.7 ± 0.8[a]
Conjugated dienes	1.9 ± 0.7[a]	2.0 ± 0.5[a]	2.1 ± 0.2[a]	2.4 ± 0.4[a]
TBARS (μmol/g)	1.4 ± 0.3[a]	1.6 ± 0.3[a]	1.2 ± 0.2[a]	1.1 ± 0.5[a]
Iodine value (g/100 g)	141.7 ± 0.7[d]	198.4 ± 0.7[b]	153.9 ± 0.8[c]	207.7 ± 0.6[a]
Saponification value (mg KOH/g)	269.4 ± 0.9[b]	118.7 ± 0.5[d]	273.5 ± 0.2[a]	142.5 ± 0.4[c]
Acid value (%)	0.6 ± 0.3[a]	0.05 ± 0.0[b]	0.9 ± 0.2[a]	0.04 ± 0.0[b]
Tocopherols (ppm)[b]	1976	1488	1120	730
Acylglycerol composition (%) of final oil				
TAG	—	88.5	—	90.2
DAG	—	8.8	—	9.0
MAG	—	2.6	—	0.8

[a]Mean ± SD of triplicate determinations from different experiments. Means sharing the same roman superscripts in a row are not significantly ($P \geq 0.05$) different from one another.
[b]Total of α-, γ-, and δ-tocopherols. Abbreviation: DHA, docosahexaenoic acid.

those of their unmodified counterparts. The higher level of oxidation in modified oils is due to a higher degree of unsaturation and a lower level of tocopherols present. All samples, both unmodified and enzymatically modified, followed an increasing trend in their CD content throughout the experiment period. As lipid peroxidation proceeds, more primary products such as hydroperoxides and conjugated dienes may be formed. The CD of modified borage oil increased from 2.0 to 15.0 after 4 d of storage under Schaal oven conditions at 60°C. However, the corresponding CD value for unmodified borage oil did not exceed 11.0. Similarly, the CD of modified evening primrose oil reached 22.7 as compared to 20.5 for unmodified evening primrose oil. The high content of CD in enzymatically modified oils may arise from their high proportions of readily oxidizable DHA as compared to those of their unmodified counterparts. Lipid radicals formed during the initial oxidation step may undergo rearrangement; thus, the methylene-interrupted feature of PUFA is lost in favor of for-

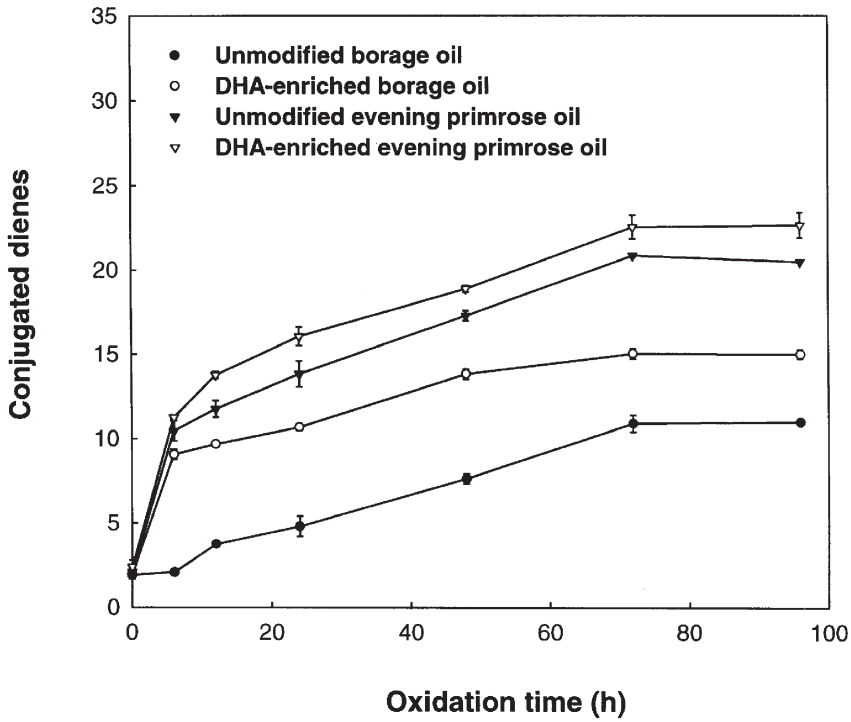

FIG. 1. Conjugated diene values of borage and evening primrose oil-based structured lipids and unmodified oils stored under Schaal oven conditions at 60°C. DHA, docosahexaenoic acid.

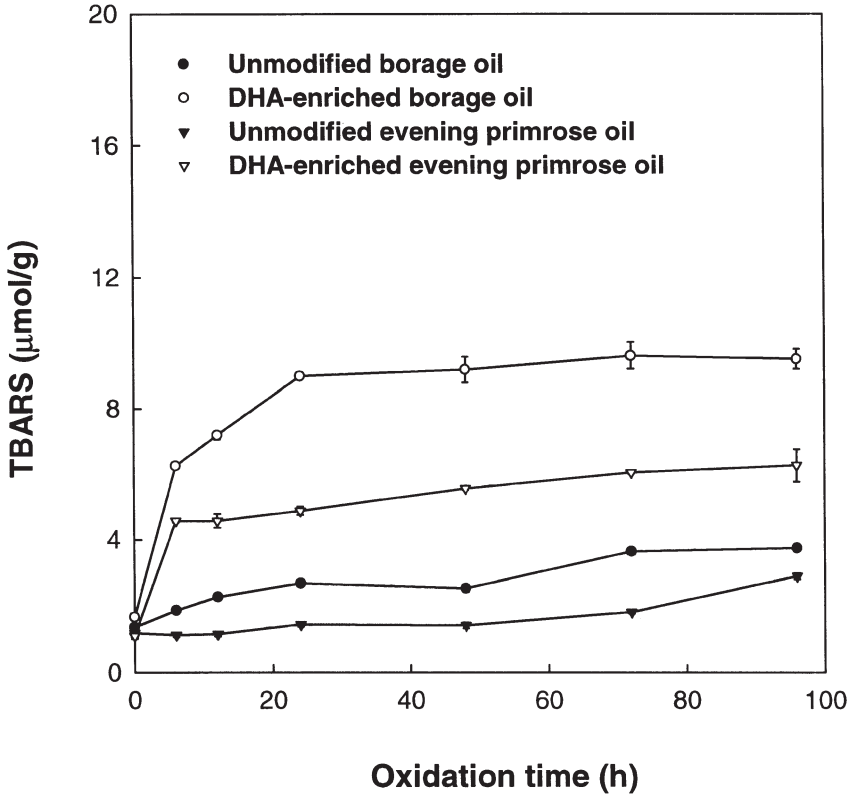

FIG. 2. TBARS values of borage and evening primrose oil-based structured lipids and unmodified oils stored under Schaal oven conditions at 60°C. For abbreviation see Figure 1.

mation of CD (2). Farmer and Sutton (23) and Jackson (24) noted that the formation of lipid hydroperoxides coincides with that of the CD upon oxidation.

The TBARS value has been used to measure the secondary products of lipid oxidation. During the early stages of oxidation, the amounts of TBARS in oxidized lipids are correlated well with CD values (25). Production of TBARS of both enzymatically modified and unmodified oils is given in Figure 2. The TBARS values increased progressively over the entire storage period. Furthermore, enzymatically modified oils had significantly higher ($P \leq 0.05$) TBARS values than those of their unmodified counterparts. The observed trends in TBARS (Fig. 2) are similar to those of CD (Fig. 1). The general increase in TBARS values during the storage period is due to the fact that as oxidation proceeds, lipid hydroperoxides break down to produce secondary oxidation products, as supported by the findings of Strange *et al*. (26). The high content of TBARS in enzymatically modified oils was due to the incorporation of high proportions of PUFA (i.e., DHA) in the oils examined. Therefore, oils modified with DHA were more susceptible to oxidation than their unmodified counterparts. It is suggested that the removal of endogenous antioxidants, including tocopherols, during processing may be partially responsible for the observed trends. A close scrunity of the results indicated that the amount of tocopherols was reduced in the modified oils (Table 2). Ferrari *et al*. (27) have suggested that decreased oxidative stability of vegetable oils during processing is due to the loss of their natural antioxidants, which is in agreement with the results of the present work.

Static headspace volatile analysis of modified oils revealed a striking increase in the number and intensity of peaks. Hexanal and propanal were monitored as indicators of lipid oxidation in oils. Hexanal is mainly formed from n-6 FA, and propanal is a major breakdown product of n-3 FA such as EPA and DHA (28). The major volatile compound detected from the original borage and evening primrose oils was hexanal, which is derived from LA (Fig. 3). Upon enrichment of these oils with DHA *via* enzymatic acidolysis, they produced both hexanal and propanal as their main volatiles during degradation. The other volatile compounds identified in enzymatically modified oils were acetaldehyde, pentanal, heptanal, and nonanal (Fig. 4). The formation of these volatiles appears to be consistent with oxidative deterioration of DHA and LA, which are present in appreciable amounts in modified oils. The absence of 2,4-decadienal, a major autoxidation product of LA, in the oxidized samples may be due to its further oxidation to hexanal (29). Medina *et al*. (30) noted that in fish muscle lipids, the levels of acetaldehyde, propanal, heptane, 2-ethylfuran, pentanal, and hexanal increased during oxidation. Shahidi and Spurvey (31) reported that formaldehyde, acetaldehyde, propanal, pentanal, and hexanal were formed during oxidation of fish muscle lipids. Neff and List

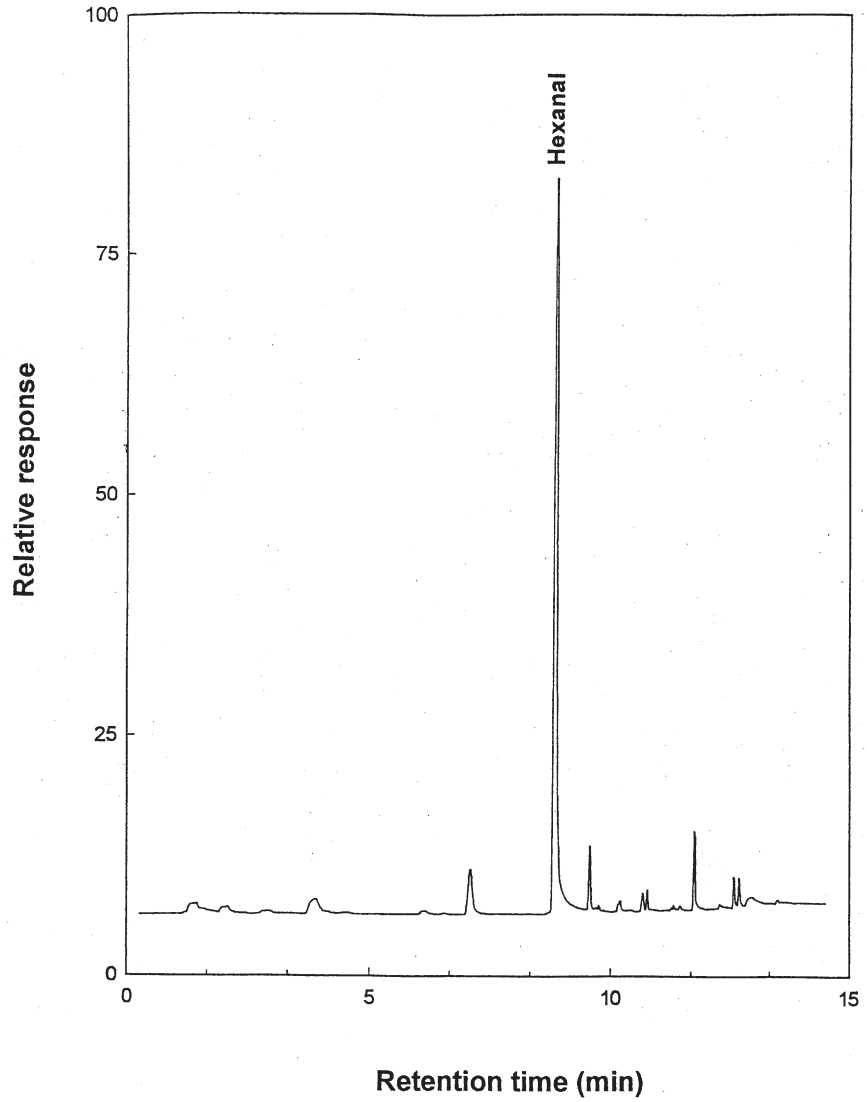

FIG. 3. Chromatogram of the headspace volatiles of unmodified borage oil after 4 d of storage at 60°C.

(32) identified LA-derived aldehydes, namely pentane, pentanal, hexanal, and heptenal, as the predominant volatiles formed following oxidation of soybean oil stored at 60°C. The amount of propanal produced in modified oils increased with the oxidation time (Fig. 5). Under similar conditions, unmodified oils showed no significant formation of propanal because of their low content of n-3 PUFA as compared to the amounts present in the modified oils. Similar increases in propanal levels during storage of fish oils (33) and fish muscle lipids (30,31) were reported. The hydroperoxides formed from α-linolenic acid (ALA) degrade to a variety of products, including propanal, ethane, and 2,4,7-decatrienal (34). Propanal is a predominant oxidation product of ALA and lipids containing a large proportion of this FA or those containing long-chain PUFA such as EPA and DHA (31).

Hexanal concentration increased with increasing oxidation time (Fig. 6). The content of hexanal produced in enzymatically modified oils was increased ($P \leq 0.05$) despite a significant reduction in the proportion of LA in the products. Modified oils still contained appreciable amounts of LA, which may have had an effect on hexanal formation. The other explanation is that propanal and other oxidation products generated in modified oils may act as pro-oxidants. Various pro-oxidants may have the ability to accelerate lipid oxidation reactions, and it is possible that secondary degradation products of hydroperoxides (volatiles) act as pro-oxidants. This might partially explain the higher concentration of hexanal that was observed in modified oils and lends further support to the findings of El-Magoli *et al*. (35), who reported that presence of lipid degradation products (volatile compounds resulting from PUFA oxidation) in the oils catalyzed their oxidation. In addition, Jung *et al*. (36) reported that deodorization of oils may increase oxidative stability because pro-oxidants such as oxidation products, moisture, MAG, and FFA remaining in the bleached oil are removed during this process.

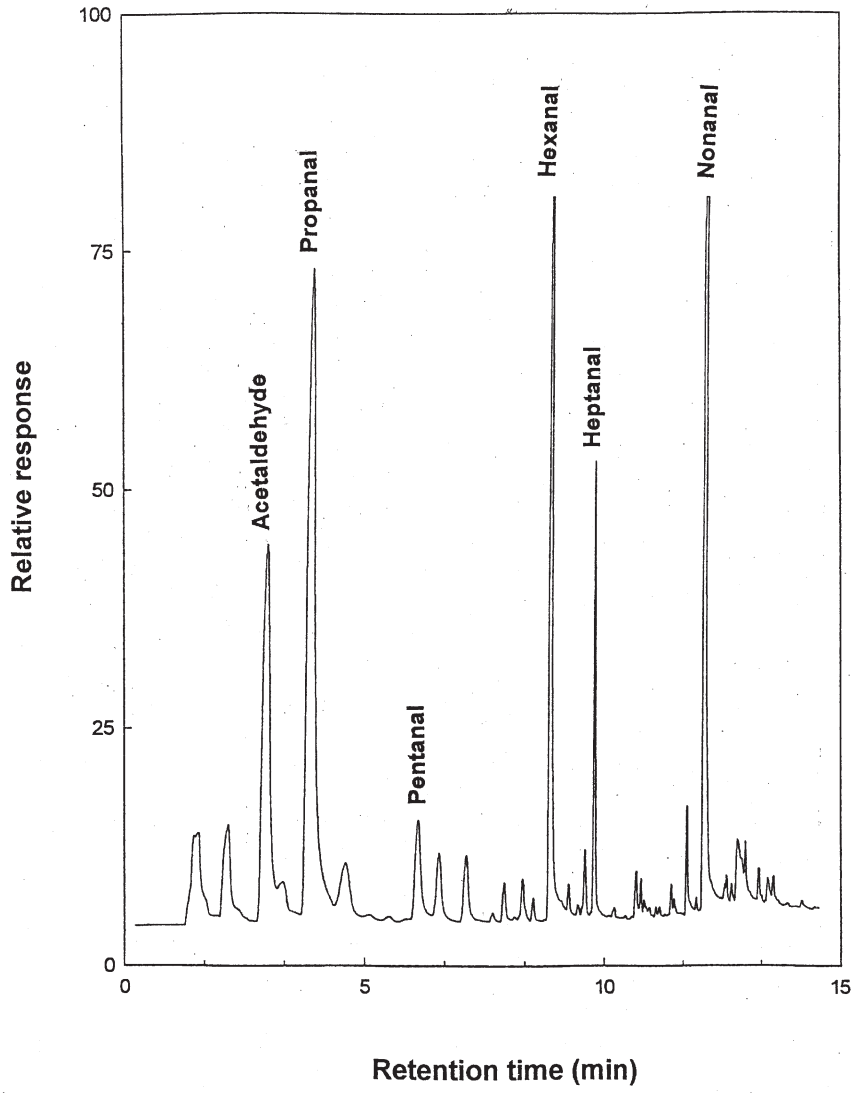

FIG. 4. Chromatogram of the headspace volatiles of modified borage oil after 4 d of storage at 60°C.

Autoxidation of n-3 FA, especially EPA and DHA, results in the formation of more 18- and 20-hydroperoxides than other hydroperoxides, which may generate propanal upon homolytic cleavage. Similarly, n-6 PUFA, especially LA, produce more of the 13-hydroperoxides as their primary product of oxidation and produce hexanal upon homolytic cleavage (37). LA, which is n-6, can be expected to produce pentane, hexanal, malondialdehyde, octanal, and 2,4-decadienal upon oxidation (38). Shahidi *et al.* (39) found a direct relationship between hexanal content and sensory scores of cooked ground pork, which contains a high amount of LA. Medina *et al.* (30) reported that headspace volatiles produced from oxidized fish muscle lipids correlated highly with TBARS values.

The changes in DBI and MBI of DHA-enriched oils as well as their unmodified counterparts were plotted against storage period (Fig. 7). As expected, there was a decline in the DBI and MBI of oils during oxidation. The extent of decrease produced was greater in the modified oils, which are more unsaturated, but this decrease was minimum for unmodified oils. The DBI decreased from 2.73 to 2.50 in DHA-enriched borage oil and from 1.63 to 1.52 in unmodified borage oil. Similarly, the MBI was also decreased from 1.85 to 1.66 in DHA-enriched borage oil and from 0.80 to 0.73 in unmodified borage oil (Fig. 7). Similar results were obtained for enzymatically modified evening primrose oil and unmodified evening primrose oil. The DBI decreased from 2.61 to 2.47 in DHA-enriched evening primrose oil and from 1.81 to 1.76 in unmodified evening primrose oil. The MBI decreased from 1.69 to 1.57 in DHA-enriched evening primrose oil and from 0.91 to 0.89 in unmodified evening primrose oil (Fig. 7). As expected from their higher degrees of unsaturation, the DBI and MBI values were significantly higher ($P \leq 0.05$) in the

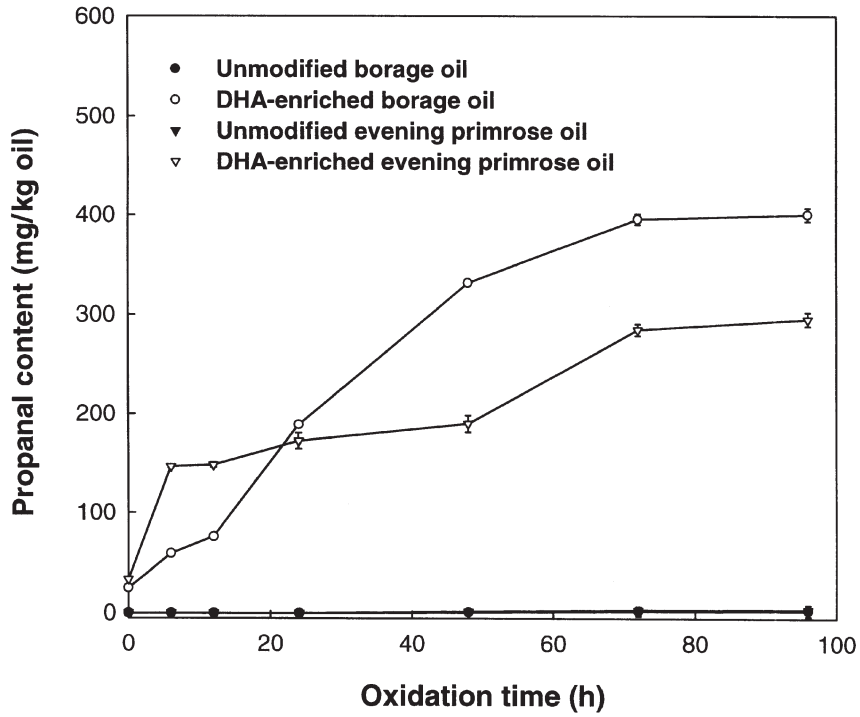

FIG. 5. Propanal contents of borage and evening primrose oil-based structured lipids and unmodified oils stored under Schaal oven conditions at 60°C. For abbreviation see Figure 1.

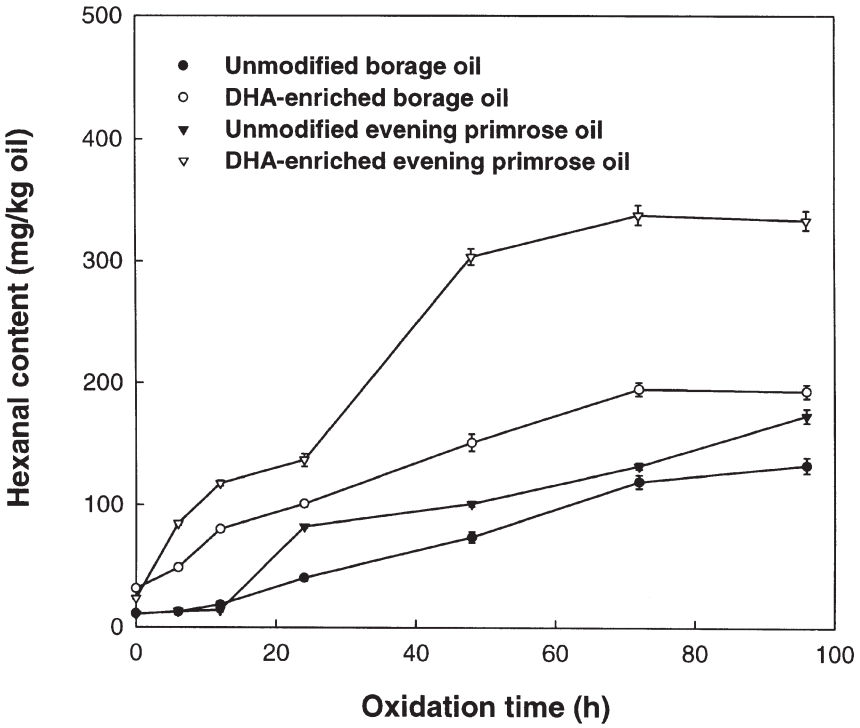

FIG. 6. Hexanal contents of borage and evening primrose oil-based structured lipids and unmodified oils stored under Schaal oven conditions at 60°C. For abbreviation see Figure 1.

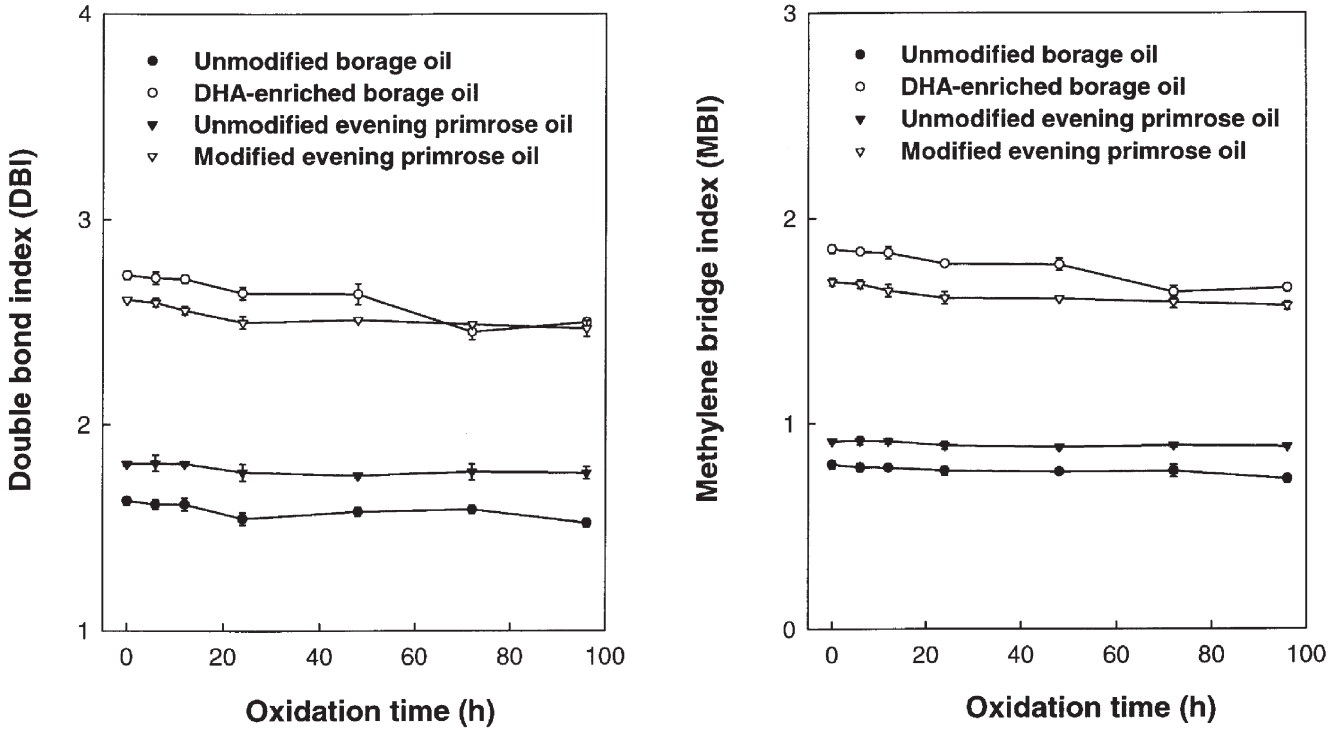

FIG. 7. Changes in double bond index (DBI) and methylene bridge index (MBI) of borage and evening primrose oil-based structured lipids and unmodified oils during storage period at 60°C. For other abbreviation see Figure 1.

enzymatically modified oils as compared with those of their unmodified counterparts. The DBI represents the number of double bonds, and MBI represents the number of bis-allylic methylene bridge positions in PUFA (21). Wagner *et al*. (20) demonstrated that the number of bis-allylic positions from which hydrogen could be abstracted by free-radical processes is the major determinant for oxidizability of cellular lipids. In studies using homogeneous solutions of purified lipids, a linear correlation existed between the number of bis-allylic positions and the oxidizability of the lipids (20,40).

To test how DBI and MBI can be used to evaluate oxidative stability of oils, regression analyses were carried out between each index and CD, TBARS, hexanal, and propanal contents of oils. Since DBI and MBI decrease while other parameters increase during oxidation, negative correlations existed between these variables. Significant negative correlations ($P \leq 0.05$) existed between data for DBI and MBI and CD values of modified oils; correlation coefficients were 0.821 for DBI and 0.843–0.866 for MBI. However, corresponding values for unmodified oils were 0.741–0.752 and 0.742–0.910, respectively (Table 3). Good negative correlations were obtained between DBI or MBI and TBARS values of oils; correlation coefficients were 0.621–0.771 for DBI and 0.677–0.791 for MBI for modified oils, and the corresponding values for their unmodified counterparts were 0.754–0.811 and 0.760–0.973, respectively (Table 3). In addition, significant negative correlations ($P \leq 0.05$) existed between DBI and MBI with hexanal content of oils; the correlation coefficients (r) were 0.728–0.842 for DBI and 0.793–0.865 for MBI for modified oils and 0.576–0.718 for DBI and 0.574–0.877 for MBI for their unmodified counterparts (Table 4). Propanal contents were also negatively correlated ($P \leq 0.05$) with those for DBI and MBI of modified oils with respective r = 0.703–0.831 and 0.751–0.853 (Table 4). Therefore, these results suggest that indicators such as DBI and MBI, representing structural characteristics of lipid molecules involved,

TABLE 3
Correlation Coefficients (r) Between Conjugated Dienes (CD) and Double Bond Index (DBI) and Methylene Bridge Index (MBI) as Well as Between TBARS and DBI and MBI of Oxidized Oils

Sample	CD vs. DBI	CD vs. MBI	TBARS vs. DBI	TBARS vs. MBI
Unmodified BO	–0.741[b]	–0.910[a]	–0.811[b]	–0.973[a]
DHA-enriched BO	–0.821[b]	–0.843[b]	–0.771[b]	–0.791[b]
Unmodified EPO	–0.752[b]	–0.742[b]	–0.754[b]	–0.760[b]
DHA-enriched EPO	–0.821[b]	–0.866[b]	–0.621	–0.677

[a]Significant at $P < 0.005$ level.
[b]Significant at $P < 0.05$ level. BO, borage oil; EPO, evening primrose oil; for other abbreviation see Table 2.

TABLE 4
Correlation Coefficients (*r*) between Hexanal Content and DBI and MBI as Well as Between Propanal Content and DBI and MBI of Oxidized Oils

Sample	Hexanal content vs. DBI	Hexanal content vs. MBI	Propanal content vs. DBI	Propanal content vs. MBI
Unmodified BO	–0.718[a]	–0.877a	—	—
DHA-enriched BO	–0.842[a]	–0.865[a]	–0.831[a]	–0.853[a]
Unmodified EPO	–0.576	–0.574	—	—
DHA-enriched EPO	–0.728[a]	–0.793[a]	–0.703[a]	–0.751[a]

[a]Significant at $P < 0.05$ level. For abbreviations see Tables 2 and 3.

have a good influence on oxidative stability of both modified and unmodified oils. However, the influence of endogenous antioxidants in the oils and their presence in various amounts that may have affected the correlation values in different cases cannot be ignored.

The results presented in this study have provided simple and reliable analytical methods to follow the oxidation of modified and unmodified borage and evening primrose oils, and enhanced our understanding of the parameters involved in the oxidation of nutritional and medicinal oils. Based on the results obtained in this study, it is recommended that the applicability of DBI and MBI as indicators of oxidation of lipids in other food systems be evaluated. Furthermore, the relationships of these indicators with sensory properties need to be established.

The ease of autoxidation of FA is proportional to the number of methylene groups between double bonds; thus, modified borage and evening primrose oils with a higher content of DHA were more prone to oxidation than their unmodified counterparts. Thus, modified oils rich in PUFA compared to unmodified oils must be protected against oxidation in order to counterbalance any harmful effects from production of oxidation products and to take advantage of their nutritional and health benefits. It is recommended that the addition of appropriate antioxidants (natural or synthetic) to the structured lipids, up to the level that would prevent oxidation of PUFA before incorporation into food, used as nutraceuticals or for other applications be examined. Incorporation of n-3 PUFA-enriched borage and evening primrose oils into foods may reduce the risk of developing certain disease conditions in humans, but such uses need to be justified using evidence gathered from animal studies and clinical trials.

ACKNOWLEDGMENTS

This research was financially supported by a research grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada to the corresponding author. The supply of algal oil from Martek Biosciences Corporation (Columbia, MD), borage oil from Bioriginal Food and Science Corporation (Saskatoon, Saskatchewan, Canada), evening primrose oil from Efamol, Inc. (Kentville, Nova Scotia, Canada), and the enzyme from Novo Nordisk Biochem North America Inc. (Franklinton, NC) is gratefully acknowledged.

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[Received March 4, 2002; accepted June 11, 2002]