Oxidative Stability of Stripped and Nonstripped Borage and Evening Primrose Oils and Their Emulsions in Water

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ABSTRACT: Oxidative stability of stripped and nonstripped borage and evening primrose oils and their emulsions in water was evaluated. The results indicated that column chromatographic techniques provide an effective means for stripping vegetable oils of their minor components. However, some minor components may be retained in the stripped oils. The minor components in borage and evening primrose oils significantly ($P < 0.05$) influenced their oxidative stability in the dark. In contrast, the behavior of endogenous antioxidants in borage and evening primrose oil-in-water emulsions, according to the "polar paradox" theory, was difficult to evaluate. Correlations existed between peroxide value (PV) and conjugated dienes (CD) ($P < 0.05$) as well as 2-thiobarbituric acid-reactive substances (TBARS) and hexanal content ($P < 0.01$) for most oils and emulsion systems. Therefore, CD and TBARS may generally be used to assess the oxidative stability of borage and evening primrose oils and their oil-in-water emulsions in addition to or in place of PV and headspace volatiles, respectively.

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KEY WORDS: Borage oil, evening primrose oil, gamma-linolenic acid, Iatroscan, oil-in-water emulsion, polar theory, stripped oil.

Special varieties of herbaceous oilseed crops are grown for the production of nutritional and medicinal oils (1). The oils from seeds of borage (*Borago officinalis*) and evening primrose (*Oenothera biennis*) are rich in gamma-linolenic acid (GLA), and thus have become major commercial sources of this essential polyunsaturated fatty acid (PUFA) (2).

Edible vegetable oils consist mainly (95%) of triacylglycerols. Nontriacyglycerols, or minor components, make up the remaining 5%. The minor components of vegetable oils are primarily composed of phospholipids, tocopherols, tocotrienols, flavonoids, carotenoids, chlorophyll, and sterols as well as free fatty acids, and mono- and diacylglycerols (3). Several classes of these components might be present in each oil and act as anti- or prooxidants (4). The behavior of antioxidants in edible oil-in-water emulsions has been explained by the "polar paradox" theory (5). This theory suggests that nonpolar lipophilic antioxidants, such as tocopherols, are more active in oil-in-water emulsions than the polar hydrophilic antioxidants, such as ascorbic acid. The opposite trend has been observed in bulk oil systems. In this dry system, the lipophilic antioxidants will remain in the oil, while the hydrophilic antioxidants will be oriented in the air-oil interface, and thus more effective in reducing or preventing oxygen accessibility for oil oxidation.

Borage and evening primrose oils contain high levels of PUFA and thus are readily susceptible to oxidation (6,7). Moreover, oxidation of nutritional and pharmaceutical emulsions affects the safety and efficiency of these products (8). Nevertheless, little is known about the oxidative stability (OS) of borage and evening primrose oils, and virtually nothing is known about the OS of these oils when stripped of their minor components or their emulsions in water.

Most of the analytical methods used to follow edible oil oxidation have limitations (9). Therefore, it has been recommended that the progress of oxidation be followed by more than one method and by measuring both the primary and the secondary oxidation products (10). It is also possible to determine the extent to which various methods agree with one another by using carefully controlled model systems. This can be achieved by calculating the correlation coefficients (11) or by employing linear regression analysis (12).

The current research was designed to: (i) evaluate the OS of stripped and nonstripped borage and evening primrose oils in bulk and emulsion systems using different analytical methods, and (ii) determine the minor components in stripped and nonstripped borage and evening primrose oils and their effect on OS of oils and emulsions.

MATERIALS AND METHODS

Materials. Cold-pressed borage oil was obtained from Bioriginal Food & Science Co. (Saskatoon, Saskatchewan, Canada). The evening primrose oil was obtained from Scotia Pharmaceuticals (Kentvill, Nova Scotia, Canada). Both oils were of high quality as indicated by their peroxide value (PV) and 2-thiobarbituric acid-reactive substances (TBARS) content (see Table 1). Tocopherols (α-, δ-, and γ-), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidyl-

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inositol, lysophosphatidylcholine, phosphatidylethanolamine (PE), phosphatidic acid (PA), lysophosphatidylethanolamine, oleic acid, monoolein, diolein, triolein, linoleic acid ethyl ester, 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (TMP), as well as sucrose and Tween 40 were obtained from Sigma Chemical Co. (St. Louis, MO). Compressed air, hydrogen, and ultra high purity helium were obtained from Canadian Liquid Air Ltd. (St. John's, Newfoundland, Canada). Activated charcoal was acquired from BDH Inc. (Tornoto, Ontario, Canada). Celite 545 was obtained from Fisher Scientific (Fair Lawn, NJ). Silicic acid powder (mesh 100) was purchased from Mallinckrodt Canada, Inc. (Point-Claire, Quebec, Canada). All other chemicals were ACS grade or better.

Preparation of minor component-stripped oils. Borage and evening primrose oils were stripped of their minor components following the method of Lampi *et al.* (12) with modifications. A chromatographic column (3.4 cm i.d. × 40 cm) was connected to a water-pump vacuum and packed sequentially with four adsorbents. The bottom layer consisted of activated silicic acid (40 g), followed by a mixture of Celite 545/activated charcoal (20 g; 1:2 w/w), a mixture of Celite 545/powdered sugar (80 g; 1:2 w/w), and activated silicic acid (40 g) as the top layer. All adsorbents were suspended in *n*-hexane. Oil (100 g) was diluted with an equal volume of *n*-hexane and passed through the chromatographic column. The solvent in the eluant (stripped oil) was evaporated under vacuum at 30°C, and traces of the solvent were removed by flushing with nitrogen. Samples of stripped and nonstripped oils were kept at −70°C for subsequent studies.

Preparation of oil-in-water emulsions. Ten percent (wt/vol) borage and evening-primrose oil-in-water emulsions were prepared as described by Frankel *et al.* (13) with modifications. Oil (5 g), distilled water (45 mL), and Tween 40 were emulsified by mixing gently for 5 min, followed by sonication at maximal power for 25 min in an ultrasonic sonicator model 6 QT (NEYO, Yucaipa, CA) filled with ice-cold water (≈1°C).

Preparation of samples for accelerated oxidation tests. Stripped and nonstripped oil samples (5 g) and oil-in-water emulsions (10 g) were used to study their OS in the dark. The sample containers were covered with aluminum foil and kept at 60°C in a forced-air Precision Oven Model 2 (Precision Scientific Co., Chicago, IL) (14).

Removing and extracting samples for OS tests. Oil and emulsion samples were removed from the oven after 24, 72, 120, and 168 h, flushed with nitrogen, covered with Parafilm, and kept at −70°C for OS tests. Prior to the tests, the oil was extracted from the emulsion (50 g) by adding methanol (50 mL) and hexane (50 mL) using a separatory funnel (13).

OS tests. The OS of stripped and nonstripped oil and oil-in-water emulsions was evaluated by determining PV (15), conjugated dienes (CD) (16), and TBARS (15), which were calculated by multiplying the absorbance readings at 532 nm by a factor of 0.145, determined from a standard line prepared using TMP as a precursor of malonaldehyde (MA) (17), and headspace volatiles, epecially hexanal, as described by Shahidi *et al.* (18).

Chemical and instrumental analysis. Fatty acid composition of the oils was analyzed according to the method described by Wanasundara and Shahidi (19). Neutral (NL), glycolipids and phospholipids (PL) were separated by silicic acid column chromatography (20). The NL and PL fractions obtained in the previous step were used to determine the concentration of NL and PL subclasses according to Du Plessis and Pretorius (21) and Imbs and Pham (22) with modifications. Fractions of NL and PL (1 μL or 10 μg) in chloroform/methanol (2:1, vol/vol) were spotted on Chromarods-SII and dried for 10 min. The rods were transferred into a developing tank containing a benzene/chloroform/acetic acid (70:30:4, vol/vol/vol) or a chloroform/methanol/water (70:30:3, vol/vol/vol) mixture for the separation of NL and PL classes, respectively. After 45 min, the rods were dried at room temperature for 10 min and incubated at 100°C for 3 min. After this period, the rods were removed from the oven and cooled to room temperature. The lipid fractions were analyzed on an Iatroscan MK-5 TLC/FID (Iatron Laboratories Inc., Tokyo, Japan). The flame-ionization detection (FID) scanning speed was 40 s/rod, the hydrogen flow rate was 160 mL/min, and the air flow rate was 2,000 mL/min. Individual lipids were identified by comparison of their retention times with those of known standards. Quantification, on a percentage basis, was achieved by utilizing external standard and TSACN computer software (S.P.E. Limited, Concord, Ontario, Canada). Pigments present in the stripped and nonstripped oil samples were monitored by measuring the absorbance at 430–460 nm for carotenoids and 550–710 nm for chlorophyll and its derivatives (23). Tocopherols' contents in the stripped and nonstripped oils were determined using a Shimadzu high-performance liquid chromatograph equipped with two LC-6A pumps, SPD-6AV, Lichrosorb Si 60 analytical column (3.2 × 200 mm, 5μ; Merck, Montréal, Québec, Canada) and an ultraviolet-visible detector (18).

Statistical analysis. All experiments and/or measurements were replicated three times. Mean ± standard deviation was reported for each case. Analyses of variance and Tukey's studentized test were performed at a level of $P < 0.05$ to evaluate the significance of differences between mean values. Linear regression analysis was used to establish the relationship between PV and CD as well as between hexanal and TBARS (24). Data presented (as %) were transformed, prior to statistical analysis, using the following formula: transformed $x = \arcsin\sqrt{x/100}$.

RESULTS AND DISCUSSION

Chemical characteristics of stripped and nonstripped borage and evening primrose oils. The chemical characteristics of cold-pressed, nonstripped borage and evening primrose oils are summarized in Table 1. Data in this table demonstrate that nonstripped oils have good OS, and that nonstripped evening primrose oil (NEPO) contained more ($P < 0.05$) primary and secondary oxidation products than nonstripped borage oil

TABLE 1
Chemical Characteristics of Nonstripped and Stripped Borage and Evening Primrose Oils

	Characteristics	NBO	SBO	NEPO	SEPO
Oxidative status	PV (meq/kg)	1.65 ± 0.27[c]	0[a]	3.16 ± 0.11[d]	1.0 ± 0.23[b]
	CD	1.60 ± 0.1[b]	0[a]	3.12 ± 0.4[d]	2.05 ± 0.05[d]
	TBARS	0.56 ± 0.1[b]	0.2 ± 0.01[a]	1.65 ± 0.2[d]	0.68 ± 0.1[c]
	Hexanal	1.92 ± 0.06[b]	0[a]	4.14 ± 0.11[d]	0[a]
Lipid classes (weight %)	NL	95.1 ± 0.1[a,b]	99.89 ± 1.1[b]	93.2 ± 0.5[a]	99.56 ± 0.89[b]
	GL	3.8 ± 0.1[b]	0[a]	5.3 ± 0.2[c]	0[a]
	PL	0.9 ± 0.1[b]	0[a]	1.8 ± 0.1[c]	0[a]
Tocopherols[b] (mg/kg)	α	0[a]	0[a]	16[b]	0[a]
	δ	52[c]	0[a]	0[b]	0[a]
	γ	659[d]	0[a]	335[c]	84[b]
	Total	711[a]	0[a]	341[c]	84[b]
Pigments absorbance	430 nm	0.07 ± 0[c]	0[a]	0.62 ± 0.01[d]	0.01 ± 0[b]
	460 nm	0.02 ± 0[a]	0[a]	0.27 ± 0[b]	0[a]
	550 nm	0[a]	0[a]	0.09 ± 0[b]	0[a]
	620 nm	0[a]	0[a]	0.05 ± 0[b]	0[a]
	670 nm	0[a]	0[a]	0[a]	0[a]

[a]Values are means of three determinations ± standard deviations. Values followed by roman superscript letters in each row are different ($P < 0.05$) from one another.
[b]Average of duplicate analysis. Abbreviations: NBO, nonstripped borage oil; NEPO, nonstripped evening-primrose oil; SBO, stripped borage oil; SEPO, stripped evening-primrose oil; PV, peroxide value; CD, conjugated diene; TBARS, thiobarbituric acid-reactive substances; NL, neutral lipids; GL, glycolipids; PL, phospholipids.

(NBO).

NL contents of NBO and NEPO were 95.1 ± 0.1 and 93.2 ± 0.5%, respectively. However, NEPO had higher amounts PL than NBO. The main tocopherols in NBO were δ- (52 ppm) and γ- (659 ppm) tocopherols, while NEPO contained α- (16 ppm) and γ- (335 ppm) tocopherols. Carotenoids and chlorophylls were present in significantly ($P < 0.05$) higher amounts in NEPO than in NBO.

Stripped borage (SBO) and evening primrose (SEPO) oils were successfully prepared using a modified multilayer column chromatographic technique. This procedure required only 2 h to strip 100 g of borage and evening primrose oils. No peroxides, CD, hexanal, pigments, tocopherols, phospholipids, or glycolipids were detected in SBO. In SEPO, OS, as shown by PV (1 meq/kg) and CD (2.05) values and secondary oxidation products, represented by TBARS (0.68 μmol/g), as well as γ-tocopherol (84 ppm), were reduced from their initial amounts of 3.16 meq/kg (PV), 3.12 (CD), 0.68 μmol/g (TBARS), and 341 ppm (γ-tocopherol) in NEPO. No hexanal, glycolipids, phospholipid, α-tocopherol, or pigments were detected in SEPO.

The fatty acid compositions of NBO, SBO, NEPO, and SEPO are given in Table 2. The results in this table indicate that NEPO and SEPO contained higher amounts ($P < 0.05$) of PUFA than NBO and SBO. The main PUFA in both oils was linoleic acid, which was present at more than 70% in SEPO and NEPO, but only at 36% in SBO and NBO. Borage oil had up to 22% GLA (18:3n-6) while evening primrose oil contained only 9% GLA. Thus, borage oil serves as a richer source of this essential fatty acid as compared to evening primrose oil. SBO and NBO had higher amounts ($P < 0.05$) of oleic acid (18:1) than SEPO and NEPO. Furthermore, low quantities of eicosenoic or gondoic (20:1), docosenoic (22:1), as well as tetracosenoic or nervonic (24:1) acids were detected in borage oil.

Thin-layer chromatography–FID (TLC–FID) combines

TABLE 2
Fatty Acid Composition (area %) of NBO, SBO, NEPO, and SEPO[a]

Fatty acid	NBO	SBO	NEPO	SEPO
16:0	11.31 ± 0.09[c]	11.00 ± 0.77[c]	6.77 ± 0.33[b]	6.54 ± 0.29[a]
18:0	4.00 ± 0.21[d]	4.28 ± 0.13[c]	1.84 ± 0.11[b]	1.80 ± 0.01[a]
18:1	16.93 ± 0.42[c]	16.54 ± 0.13[c]	8.67 ± 0.28[b]	8.31 ± 0.71[a]
18:2	36.34 ± 1.33[a]	36.96 ± 0.11[a]	73.55 ± 1.81[b]	75.75 ± 0.27[b]
18:3n-6	22.05 ± 0.92[c]	22.28 ± 1.26[c]	9.16 ± 0.58[b]	7.60 ± 0.75[a]
20:1	4.58 ± 0.03[a]	4.68 ± 0.02[a]	—	—
22:1	2.85 ± 0.05[b]	2.59 ± 0.01[a]	—	—
24:1	1.85 ± 0.07[b]	1.67 ± 0.01[a]	—	—

[a]Values are means of three determinations ± standard deviations. Values followed by roman superscript letters in each row are different ($P < 0.05$) from one another. See Table 1 for abbreviations.

TABLE 3
Neutral Lipids Composition (%), and Phospholipid Composition (% of total phospholipids) of NBO and NEPO[a]

Lipid classes	NBO	NEPO
Neutral lipids		
Steryl esters	0.29 ± 0.06[a]	0.58 ± 0.08[b]
Triacylglycerols	98.01 ± 0.05[a]	97.75 ± 0.08[a]
Free fatty acids	0.30 ± 0.01[a]	0.64 ± 0.05[b]
Diacylglycerols	0.47 ± 0.06[a]	0.33 ± 0.08[a]
Monoacylglycerols	0.67 ± 0.05[a]	0.39 ± 0.01[a]
Phospholipids		
Phosphatidic acid	20.5 ± 0.8[a]	61.42 ± 2.67[b]
Phosphatidylethanolamine + phosphatidylserine	33.11 ± 1.0[b]	5.37 ± 1.20[a]
Phosphatidylinositol	5.21 ± 0.33[a]	11.18 ± 1.92[b]
Lysophosphatidylethanolamine	3.22 ± 1.44[b]	0.17 ± 0.08[a]
Phosphatidylcholine	35.18 ± 1.15[b]	11.81 ± 1.49[a]
Lysophosphatidylcholine	0.78 ± 0.05[a]	10.04 ± 0.81[b]

[a]Values are means of three determinations ± standard deviations. Values followed by roman superscript letters in each row are different ($P < 0.05$) from one another. See Table 1 for abbreviations.

the efficacy of TLC separation and quantification by FID. Table 3 shows that NEPO oil has more ($P < 0.05$) free fatty acids than NBO, while the latter oil contained more ($P < 0.05$) mono- and diacylglycerol than the former one. The main phospholipid subclasses in NBO were PC and PE, together with PS, while the main phospholipid subclasses in NEPO were PA and PC (Table 3).

OS of SBO, NBO, SEPO, NEPO, and their oil-in-water emulsions. Based on PV obtained during the oxidation in the dark (Table 4), NBO and nonstripped borage oil emulsion (NBE) were more ($P < 0.05$) stable than their corresponding SBO and stripped borage oil emulsion (SBE). Similarly, NEPO and nonstripped evening primrose oil emulsion (NEPE) were more stable ($P < 0.05$) than SEPO and stripped evening primrose oil emulsion (SEPE). Meanwhile, NBO and NBE were more stable ($P < 0.05$) than NEPO and NEPE.

The better OS of NBO, NBE, NEPO, and NEPE compared to OS of their corresponding stripped samples may be, in part, attributed to the presence of minor components such as tocopherols and specific phospholipids in both oils. It is well known that tocopherols contribute to the OS of edible oils (25,26). However, only some phospholipids (PC and PE) have been shown to be effective antioxidants in edible oils (27). NBO and NBE were more stable than NEPO and NEPE due to the presence of higher amounts ($P < 0.05$) of tocopherol as well as PC and PE.

Free radical chain reactions occurring during autoxidation of oils may be interrupted by tocopherols, thus protecting oils and their emulsions from oxidation. This may occur by scavenging, i.e., removing the dissolved oxygen in the system, or by reacting with the generated free radicals and terminating the propagation step (25). In both cases, tocopherols are gradually consumed during the induction period. Thereafter, oils oxidize more rapidly and the end of the induction period can be determined by a dramatic increase in PV (27). This might explain the sudden increase of PV for all samples examined.

Emulsions of borage and evening primrose oils in water were physically stable during storage at 60°C for 168 h. No creaming, flocculation, coalescence, or oil separation was observed in the emulsions examined. These criteria may be used

TABLE 4
PV and CD Values of SBO, NBO, SEPO, NEPO, and Emulsions and Their Correlation[a]

	PV (meq/kg)					CD					Correlation	
Oil	0	24	72	120	168	0	24	72	120	168	*r*	*P*
SBO	0[a]	7.0 ± 1.3[c]	102.7 ± 3.3[e]	384 ± 7.8[c]	487.3 ± 28.8[e]	0[a]	4.5 ± 0.0[a]	12.5 ± 0.1[c]	35.9 ± 0.2[d]	68.2 ± 2.1[d]	0.98	$P < 0.01$
NBO	1.65 ± 0.27[d]	4.3 ± 0.1[b]	18.9 ± 1.9[a]	60.7 ± 3.9[a]	141.3 ± 4.0[b]	1.6 ± 0.1[b]	7.0 ± 0.4[c]	10.2 ± 0.0[b]	20.1 ± 0.5[b]	20.1 ± 0.5[a]	0.93	$P < 0.01$
SBE	0[a]	28.4 ± 0.1[e]	154.0 ± 8.7[f]	538.5 ± 9.8[e]	567.4 ± 22.6[f]	0[a]	4.3 ± 0.2[a]	8.4 ± 0.3[a]	55.2 ± 0.3[g]	69.9 ± 0.7[d]	0.98	$P < 0.01$
NBE	1.65 ± 0.27[d]	3.2 ± 0.1[a]	22.9 ± 1.4[b]	60.5 ± 3.4[a]	96.0 ± 3.1[a]	1.6 ± 0.1[b]	5.8 ± 0.8[b]	13.9 ± 0.8[e]	12.8 ± 0.3[a]	24.3 ± 4.5[b]	0.82	$P < 0.01$
SEPO	1.0 ± 0.23[c]	45.0 ± 1.8[f]	250.3 ± 3.7[h]	745.2 ± 0.2[f]	797.8 ± 35.6[g]	2.1 ± 0.1[c]	8.0 ± 0.1[f]	18.5 ± 0.2[g]	52.8 ± 0.3[f,g]	86.9 ± 2.3[f]	0.96	$P < 0.01$
NEPO	0.33 ± 0.17[b]	23.8 ± 0.8[d]	65.1 ± 1.6[c]	151.9 ± 6.7[b]	393.7 ± 18.5[c]	3.1 ± 0.4[d]	9.4 ± 0.3[g]	13.06 ± 0.2[d]	22.5 ± 0.8[c]	54.2 ± 0.5[c]	0.95	$P < 0.01$
SEPE	0.98 ± 0.06[c]	120.5 ± 5.1[h]	173.1 ± 17.3[g]	553.5 ± 31.2[c]	901.8 ± 15.6[h]	2.1 ± 0.1[c]	7.5 ± 0.2[e,d]	18.9 ± 0.3[g]	38.6 ± 0.1[d]	74.4 ± 4.9[e]	0.99	$P < 0.01$
NEPE	3.16 ± 0.11[c]	52.2 ± 0.4[g]	92.7 ± 2.8[d]	422.2 ± 21.9[d]	473.5 ± 3.6[e]	3.1 ± 0.4[d]	13.5 ± 0.3[h]	15.3 ± 0.8[f]	40.5 ± 1.0[e,d]	87.7 ± 2.11[f]	0.93	$P < 0.01$

[a]Values are means of three determinations ± standard deviations. Values followed by roman superscript letters in each row are different ($P < 0.05$) from one another. Abbreviations: SBE, stripped borage oil emulsion; NBE, nonstripped borage oil emulsion, SEPE, stripped evening primrose oil emulsion; NEPE, nonstripped evening primrose oil emulsion; *r*, correlation coefficient; *P*, level of significance. See Table 1 for other abbreviations.

TABLE 5
TBARS and Hexanal Content of Stripped and Non-Stripped Borage and Evening Primrose Oils and Their Correlation[a]

	TBARS (μmol MA equivalents/g)					Hexanal (mg/kg)					Correlation	
Oil	0	24	72	120	168	0	24	72	120	168	*r*	*P*
SBO	0.2 ± 0.0^{a}	5.2 ± 0.1^{e}	8.4 ± 0.3^{e}	11.5 ± 1.3^{f}	18.4 ± 0.4^{h}	0^{a}	14.6 ± 1.2^{c}	90.4 ± 5.0^{d}	286.3 ± 8.6^{d}	988.7 ± 33.4^{d}	0.84	$P < 0.01$
NBO	0.6 ± 0.1^{b}	0.7 ± 0.0^{a}	1.7 ± 0.1^{a}	1.8 ± 0.0^{a}	2.0 ± 0.0^{a}	0.2 ± 0.0^{b}	1.9 ± 0.3^{a}	5.4 ± 0.1^{a}	13.1 ± 0.1^{a}	40.9 ± 0.2^{a}	0.46	$P > 0.05$
SBE	0.2 ± 0.0^{a}	4.7 ± 0.3^{e}	7.4 ± 0.5^{f}	9.6 ± 0.1^{e}	13.7 ± 0.5^{g}	0^{a}	14.7 ± 1.1^{c}	228.7 ± 0.4^{f}	693.6 ± 5.0^{g}	1309 ± 0.3^{e}	0.75	$P < 0.01$
NBE	0.6 ± 0.1^{b}	1.5 ± 0.1^{b}	1.8 ± 0.2^{a}	2.5 ± 0.0^{b}	3.6 ± 0.1^{b}	0.2 ± 0.0^{b}	1.9 ± 0.0^{a}	11.0 ± 0.9^{b}	35.9 ± 0.7^{b}	187 ± 1.5^{b}	0.78	$P < 0.01$
SEPO	0.7 ± 0.0^{c}	3.6 ± 0.2^{d}	5.8 ± 0.3^{e}	9.7 ± 0.7^{e}	12.1 ± 0.1^{e}	0^{a}	37.7 ± 0.9^{f}	88.0 ± 2.3^{d}	670.8 ± 17.9^{f}	1299.8 ± 7.3^{e}	0.80	$P < 0.01$
NEPO	1.7 ± 0.1^{d}	3.2 ± 0.3^{d}	3.6 ± 0.2^{d}	6.1 ± 0.2^{d}	7.6 ± 0.1^{d}	0.4 ± 0.0^{c}	9.8 ± 0.2^{b}	23.2 ± 0.1^{c}	69.7 ± 0.8^{c}	517.8 ± 22.5^{c}	0.66	$P < 0.02$
SEPE	0.7 ± 0.0^{c}	8.3 ± 0.3^{f}	2.9 ± 0.1^{c}	9.7 ± 0.2^{e}	10.2 ± 0.1^{f}	0^{a}	25.2 ± 0.0^{e}	794.2 ± 18.5^{g}	697.9 ± 19.9^{g}	2552.6 ± 72.3^{g}	0.74	$P < 0.01$
NEPE	1.7 ± 0.1^{d}	2.2 ± 0.1^{c}	2.7 ± 0.1^{b}	3.7 ± 0.2^{c}	5.4 ± 0.1^{c}	0.4 ± 0.0^{c}	17.1 ± 1.9^{d}	168.2 ± 3.3^{e}	484.98 ± 15.7^{e}	2446.9 ± 0.8^{f}	0.78	$P < 0.01$

[a]Values are means of three determinations ± standard deviations. Values followed by roman superscript letters in each row are different ($P < 0.05$) from one another. See Tables 1 and 4 for abbreviations.

as indicators of emulsion stability (28). Table 4 demonstrates that NBE was generally more stable ($P < 0.05$) than NBO during the storage, as reflected in their PV, except after 72 h, where NBO was more stable ($P < 0.05$). Moreover, no differences ($P > 0.05$) were observed between the two samples of NBO and NBE after 120 h. This fluctuation in emulsion stability might be attributed to the change in pH during the storage, as no buffer was used in the emulsions preparations, and because antioxidant activity of tocopherols in emulsions is affected by the pH (29).

The higher OS of the NBE at certain storage points should be addressed by consideration of the well-known "polar paradox" theory or "interfacial" phenomenon (5). In contrast, the PV of NEPE were higher ($P < 0.05$) than the PV of NEPO. Thus, the observed trend with consideration of tocopherol content of the oil and emulsion cannot be readily explained by the "polar paradox" theory.

Oxidation of methylene-interrupted lipid dienes can cause a shift in the double-bond positions, which can be reflected by the formation of CD (14). Table 4 shows that NBO and NEPO oils and their emulsions were more stable than their corresponding stripped oil and emulsions. Moreover, NBE was more stable ($P < 0.05$) than NBO after certain storage times, while NEPE was less stable than NEPO. These results follow a similar trend when compared to PV, and thus a strong correlation ($r > 0.7$, $P < 0.01$) existed between the PV and CD of each sample (Table 4). This suggests that CD can be used, in addition to or in place of PV, to assess the OS of SBO, NBO, SEPO, and NEPO and their oil-in-water emulsions.

Secondary oxidation products of SBO, NBO, SEPO, and NEPO were determined by examining TBARS and headspace volatiles, mainly hexanal. The determination of TBARS is based on color intensity of the reaction between TBA and secondary oxidation products of PUFA, mainly MA. The TBARS expressed as μmol of MA equivalents per g of oil or emulsion were lower than those of their corresponding stripped samples (Table 5), which may be due to the presence of minor components such as tocopherols and phospholipids. Phospholipids effectively inhibited the formation of TBARS in a salmon oil model system (27). TBARS values of SBO and NBO and their emulsions were higher ($P < 0.05$) than those reported for SEPO and NEPO. The most likely explanation is that borage oil contains higher amounts ($P < 0.05$) of linolenic acid than evening primrose oil. PUFA containing three or more double bonds can produce significant amounts of TBARS, while smaller amounts are formed from linoleate (14). A sudden decrease in TBARS of SEPO after 72 h cannot be easily explained.

Determination of headspace volatiles is a very effective method to evaluate the OS of edible oils. The major volatile generated during the autoxidation of SBO, NBO, SEPO, and NEPO and their oil-in-water emulsions was hexanal. Hexanal is the major volatile produced from the oxidation of n-6-PUFA (18). Meanwhile, 2,4-heptadienal and pentanal were detected in relatively small amounts. The formation of pentanal from n-6-PUFA can be explained by thermal decomposition of hexanal or by loss of formaldehyde, while 2,4-heptadienal may be formed from autoxidation of linolenic acid (30). Hexanal production increased gradually in all samples throughout the storage period (Table 5). However, the oils and emulsions with higher amounts of tocopherols contained less hexanal than the oils and emulsions with low tocopherol concentrations. The role of tocopherols in inhibiting hexanal formation has previously been reported (13,18).

Table 5 summarizes the linear relationships between hexanal content and TBARS. Results in this table demonstrate the presence of strong correlations ($r > 0.7$, $P < 0.01$) between the contents of hexanal and TBARS of SBO, SEPO, SBE, NBE, and NEPE. Moreover, good correlations ($r > 0.6$, $P < 0.02$) existed between these two methods for NEPO. However, no correlation ($r < 0.5$, $P > 0.05$) existed between the above two indicators for NBO. Although values of TBARS provide an estimate of the content of a variety of secondary oxidation products, these may also be affected by minor components in the samples. The exact reasons for this lack of correlation remain elusive. Nonetheless, TBARS and headspace volatiles may be used interchangeably to monitor the oxidation of the samples examined, except for NBO.

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