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Antioxidant Protection of Bulk Fish Oils by Dispersed Sugars and Polyhydric Alcohols

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Selected sugars (fructose, sucrose, or raffinose) and polyhydric alcohols (sorbitol or mannitol) were equilibrated directly with bulk fish oil (10% by weight, excess) and exposed to fluorescent lighting (2550 Lx) for 24 h at 5 °C. Data for room temperature-equilibrated samples revealed that polyols functioned as antioxidants in fish oil. Increased times and temperatures of equilibration (to 90−110 °C, 1−2 mmHg, to 2 h) greatly enhanced the antioxidant activity of polyols in fish oil exposed to light. Under accelerated oxidation conditions (60 °C) in the dark, dispersed sorbitol in bulk fish oil greatly suppressed the peroxide value, primarily by chelating transition metals, while fructose showed a limited antioxidant activity. Sugars with a lower molecular weight and smaller numbers of equatorial OH groups exhibited a higher rate of permeation of sugars into fish oil triacylglycerols and hence rendered greater antioxidant activities. The treatment of bulk fish oils with polyols and then using the oils in the preparation of emulsions greatly reduced their antioxidant activities as compared to those observed for treated bulk oils. The introduction of polyols dissolved in propylene glycol into bulk fish oils at 90 °C (0.025% polyol, 0.25 h of equilibration) provided a similar antioxidant activity to that imparted by the introduction of polyols into the oil by equilibrating excess polyols (10% by weight) with them at 90−110 °C for 2 h. However, regardless of the method of the introduction of polyols to bulk fish oil, an elevated temperature (90 °C) exposure during fish oil treatment was required to induce a notable antioxidant activity.

KEYWORDS: Sugar; polyhydric alcohols; antioxidant activity; bulk fish oil; lipid oxidation; volatile compounds

INTRODUCTION

Earlier studies have indicated that transition metal chelation and free radical-scavenging mechanisms account, in part, for the antioxidant properties of sugars and polyhydric alcohols when added singly to aqueous phases of fish oil emulsions. However, when polyols and phenolic antioxidants were combined, limited additive effects were observed. Thus, synergistic hydroxyl radical-scavenging or transition metal chelation was not by observed when polyols were combined with phenolic antioxidants [butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and α-tocopherol] at or near the oil−water interface in fish oil emulsions.

These observations were attributed to the hindrance of the H-atom donating capabilities of the phenolic antioxidants caused by H-bonding of sugars and polyhydric alcohols (*1*, *2*) at or near the oil−water interface. As a result, the establishment of an H-bond network between polyols and phenolic antioxidants was proposed to account for the net reduction of antioxidant activities for the antioxidant combinations as compared to those that might have been expected for either combinations of phenolic antioxidants and polyols or individual phenolic antioxidants in the fish oil emulsions.

Because of the interference of polyols introduced into the aqueous phase of emulsions with phenolic antioxidant functionality, the approach of using polyols in emulsions appears to provide few opportunities for improving the oxidative stability of commercial fish oil emulsions beyond those provided by the existing technologies (*3*−*5*). However, other patent literature (*6*) suggests that an alternative polyol treatment approach might provide beneficial stabilizing effects to fish oils.

In a patent issued to Epstein et al. (*7*), it was claimed that a treatment involving admixing of soybean oil directly with polyhydroxy substances (i.e., glycerol, diethylene glycol, mannitol, or sorbitol) in a nonoxidizing atmosphere under reduced pressure at 216.6−260 °C suppressed the development of beany and fishy flavors in the finished oils. The conditions of the process were claimed to be similar to those encountered in commercial edible oil deodorization processing. Epstein et al. (*7*) theorized that polyhydroxy compounds somehow reacted with the double bond system of the carotenoids or other components of soybean oil to provide reaction products or conditions, which were responsible for the suppression of beany−fishy off-flavors in the treated soybean oils.

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Yasufuku et al. (*6*) later obtained a patent for a process that included a multistage molecular distillation (0.1−50 mm Torr; 100−300 °C) of mixtures containing a polyhydric alcohol (e.g., glycerol), a monoacylglycerol (e.g., monooleylglycerol, a diol), and crude fish oil to produce refined, nearly odor-free fish oil for use in margarines. Yasufuku et al. (*6*) suggested that polyhydric substances facilitated the removal of amine or ketone precursors with fishy odors from fish oils upon molecular distillation.

Surprisingly, neither the chemical mechanisms nor the technological basis for flavor suppressions described by Epstein et al. (*7*) and Yasufuku et al. (*6*) appear to have been investigated any further. Therefore, the purpose of this study was to investigate the chemical basis of polyol-induced off-flavor suppression in bulk fish oils and to develop means to utilize it for enhancing the flavor stability of bulk fish oils and fish oils present in foods.

MATERIALS AND METHODS

Materials. Commercial fish oil (RBU-D code 548, SPMO brand nondeodorized menhaden oil—refined, bleached, and undeodorized dietary grade; Omega Protein, Reedville, VA) was obtained and stored at 5 °C until used (less than 6 months). 1,2-Propanediol (propylene glycol), α-tocopherol, pyridine, sorbitol, fructose, raffinose, mannitol, lactose, and sucrose were purchased from Sigma Chemical Company (St. Louis, MO). BHT and TBHQ were donated by Eastman Chemical (Kingsport, TN). Chloroform-D (D, 99.8%) was obtained from Cambridge Isotope Laboratories (Andover, MA). All other chemicals were reagent grade or better and used without any further purification.

Preparation of Vacuum Steam-Deodorized Fish Oils. Batches (1000 mL) of commercial menhaden oil were vacuum-deodorized at 150 ± 2 °C at an absolute pressure of 2 ± 1 mmHg for 4 h using a batch type laboratory deodorization apparatus (*8*, *9*).

Direct Equilibration Introduction of Sugars, Polyhydric Alcohols, and Phenolic Antioxidants into Bulk Fish Oils. Sugars and polyhydric alcohols (10% by weight) or TBHQ (200 ppm) were introduced into deodorized fish oil through mixing; then, the pressure was reduced to 1 mmHg, and the temperature was elevated to 90 °C and held there for 1 h while stirring with a magnetic stirrer. Then, the temperature was cooled to 21 °C, and the pressure was raised to ambient while introducing a stream of nitrogen.

Introduction of Antioxidants into Fish Oil via Propylene Glycol Solutions. A stock solution of either sorbitol or fructose in propylene glycol (25% by weight) was prepared by combining ingredients and warming to 90 °C using a heating mantle and magnetic stirring. After the solutions reached 90 °C, they were held for 15 min before heating was discontinued, and the solution was cooled to ambient temperature (21 °C) in about 45 min. The samples were then held for use in preparing fish oil samples for antioxidant efficacy trials.

To prepare fish oil samples, an appropriate amount (weight) of either sorbitol or fructose stock solution in propylene glycol was added to each fish oil sample to yield either 0.01, 0.025, 0.050, or 0.1% of the polyol in the fish oil. To maintain a uniform final concentration of 0.3% propylene glycol in each treated fish oil sample, an appropriate amount of propylene glycol also was added to compensate for that occupied by the polyol in the propylene glycol solution added.

Analysis of Bulk Fish Oil for Sugars and Polyhydric Alcohols. Fish oil samples were scanned with a Nicolet 740 Fourier Transform Infrared (FT-IR) Spectrometer equipped with a TGS detector (Nicolet Analytical Instruments, Madison, WI). Spectra (% transmittance) of treated and untreated samples were obtained using a 0.1 mm FT-IR sealed cell with NaCl windows (Perkin-Elmer Corp, Norwalk, CT).

For nuclear magnetic resonance (^{1}H NMR) analyses, fish oil samples (2.25 g) were mixed with 0.25 g of deuterated chloroform ($CDCl_3$), and spectra were obtained with a Bruker Aspect 3000 ^{1}H NMR equipped with an off-line data processing station (Bruker Spectrospin, Burlington, ON, Canada) located in the National Magnetic Resonance Facility at the University of Wisconsin—Madison.

Preparation of Fish Oil Emulsions. Fish oil emulsions were generally modeled after those described by Antrim and Tylor and Hsieh and Regenstein (*4*, *10*). When sugars or polyhydric alcohols were introduced, an equal weight amount of polyol was substituted for that amount of water in an emulsion, and they were dissolved in distilled water before initiating emulsification.

Conditions for Accelerated Oxidation and Method for Indexing Oxidation in Samples. Bulk fish oil or emulsions were exposed to lighted conditions (fluorescent, daylight type; approximately 2550 Lx) at 5 °C for 24−30 h. Samples (100 g) were layered in aluminum pans, covered with transparent polymer wrap, and placed under the lighting. The progress of oxidation was monitored by determining peroxide values using the procedure described by Buege and Aust (*11*). Standard deviations of means were also calculated for these data.

Aroma and Flavor Assessments of Fish Oil Emulsions. Bulk fish oil or emulsions were equilibrated to 21 °C and were assessed for aromas in an odor-free room immediately upon opening of the polymer films covering samples in aluminum pans. Fish oil emulsions were not assessed for flavors because of taste interference from the emulsifier employed. All assessments were performed by the author and from five to eight other experienced laboratory personnel who were familiar with the aromas of fish oils. Consensus assessments were developed for descriptive and intensity attributes of the samples.

Quantitative Analysis of Volatile Compounds in Bulk Fish Oil or Emulsion Samples. Volatile compounds in fish oil emulsions were quantitatively measured using the dynamic headspace procedure described by Olafsdottir et al. (*12*).

Headspace volatiles in bulk fish oil or emulsions were exhaustively purged from samples by sweeping with nitrogen. Volatile compounds were collected on Tenax GC (60/80 mesh, Alltech, Deerfield, IL), and subsequently, each was eluted from individual Tenax GC traps with ca. 1 mL of redistilled diethyl ether (Mallinckrodt, Paris, KY) into a concentrate tube (Laboratory Research Co., Lonita, CA). Each isolate was then concentrated for gas chromatography analysis under a slow nitrogen stream to about 10 μL at ambient temperature.

Concentrated isolates were separated by capillary column gas chromatography using a Varian 3400 gas chromatograph (Varian Associates Inc., Sunnyvale, CA) equipped with a flame ionization detector. A Carbowax 20M (60 m × 0.32 mm i.d.) fused silica capillary column (Supelco, Bellefonte, PA) operated with helium as the carrier gas was employed. The column was held at 50 °C for 1 min and then was programmed from 50 to 220 °C at a rate of 4 °C/min. The injector unit was programmed from 35 to 220 °C at a rate of 100 °C per min and then was held at 220 °C. The detector temperature was maintained at 230 °C.

The coincidence of retention indices (I_E) (*13*) for unknown peaks with those of volatile compounds identified in cod liver oil by Karahadian and Lindsay (*14*) was used for assignment of identities of compounds.

Percent changes of volatile compounds in treated samples as compared to appropriate controls were calculated from concentrations measured in the samples. When a volatile compound in the treated sample decreased in concentration as compared to the control, the following formula was used

$$\%\ \text{change}\ (-) = (\text{ppb of the volatile compound in the treated sample/ppb of the volatile compound in the control sample}) - 1 \times 100$$

When a volatile compound in the treated sample increased in concentration as compared to the control, the following formula was used

$$\%\ \text{change}\ (+) = (\text{ppb of the volatile compound in the control sample/ppb of the volatile compound in the treated sample}) - 1 \times 100$$

Table 1. Influence of Room Temperature (21 °C) Equilibration of Sugars and Polyhydric Alcohols (10% by Weight) with Deodorized Bulk Fish Oil for 2 h at 1 mmHg on the Oxidative Stability of the Fish Oils Subsequently Held under Light (2550 Lx) for 24 h at 5 °C

	antioxidant activity	
bulk fish oil treatment	peroxide value (μM hydroperoxide per mg fish oil)	change relative to control (%)
control (no polyols added)	5.9[a]	*b*
+ fructose	5.8	1.7 (–)[c]
+ sorbitol	5.7	3.4 (–)
+ raffinose	5.5	6.8 (–)
+ sucrose	5.6	5.1 (–)
+ mannitol	5.9	0

[a] Average of four replicate analyses. Standard deviation <±0.3 for all determinations. [b] Not applicable; no polyol was added to control samples. [c] Extent and direction of change.

Analyses were performed on duplicate samples. Standard deviations for the means were calculated.

RESULTS AND DISCUSSION

Demonstration of Antioxidant Activity of Sugars and Polyhydric Alcohols in Bulk Fish Oils. Initial trials were carried out to test the hypothesis that sugars and polyhydric alcohols contacted with bulk fish oils under mild conditions could provide some degree of protection, presumably through an activity afforded by residual polyols situated at the air/oil interface (*15*−*17*). The data in **Table 1** for bulk fish oils equilibrated with excess selected sugars and polyhydric alcohols at 21 °C showed some suppression of oxidation by all polyols (1.7−6.8%) as compared to the control sample. Only mannitol did not show an antioxidant effect under these experimental conditions. Thus, the data revealed that a generally sufficient residual polyol was retained in the bulk fish oils at room temperature to provide measurable antioxidant activity during subsequent exposure to light (2550 Lx) for 24 h at 5 °C. However, the extent of oxidation suppression (<6.8%; **Table 1**) by polyols in bulk fish oil prepared at 21 °C as compared to the control sample was somewhat less than that observed when sugars and polyols were introduced at 16% of the aqueous phase of model emulsions (11−18% suppressions as compared to controls).

The initial observations led to trials to determine if greater antioxidant activity could be achieved for polyols by employing conditions enhancing their dispersion in fish oils. Data showing the influence of increasing temperatures for direct equilibration of excess sorbitol (10% by weight) with deodorized bulk fish oils for 2 h at 1 mmHg upon subsequent oxidative stability of bulk fish oil are shown in **Table 2**. These data revealed that as the temperature of equilibration increased, a progressively greater antioxidant activity was obtained, especially at the upper temperature (90−110 °C at 1−2 mmHg), which yielded a 36% reduction in peroxide values as compared to the appropriate control.

An upper limit elevated temperature (90−110 °C) had been arbitrarily chosen to enhance the dispersion of polyols in fish oil in an attempt to avoid potential degradation, especially that of fructose. However, on the basis of conditions of deodorization and distillation described by Epstein et al. and Yasufuku et al. (*6*, *7*), it seems likely that higher introduction temperatures, especially with shorter contact times, could be used to disperse the more stable polyols in fish oils. Pelura and Chang and Karahadian and Lindsay (*8*, *18*) have stated that deodorization temperatures between 150 and 175 °C under vacuum (<1 mmHg) yield suitable fish oils without extensive lipid degradation and polymerization.

Table 2. Effect of Various Temperatures of Equilibration of Excess Sorbitol (10% by Weight) with Deodorized Fish Oil for 2 h at 1 mmHg upon the Oxidative Stability of Fish Oils Subsequently Held under Light (2550 Lx) for 24 h at 5 °C

	antioxidant activity	
bulk fish oil treatment	peroxide value (μM hydroperoxide per mg fish oil)	change relative to control (%)
	25 °C set	
control	5.9[a]	*b*
+ sorbitol	5.7	3.4 (–)[c]
	40 °C set	
control	5.4	
+ sorbitol	5.2	3.7 (–)
	60 °C set	
control	5.5	
+ sorbitol	4.2	22.2 (–)
	90–110 °C set	
control	5.6	
+ sorbitol	3.6	36.0 (–)

[a] Average of four replicate analyses. Standard deviation <±0.3 for all determinations. [b] Not applicable; sorbitol was not added to control samples. [c] Extent and direction of change.

Because the equilibration time (2 h; **Table 2**) also had been initially arbitrarily chosen, trials were conducted to determine the influence of equilibration time at 90 °C and 1 mmHg upon the resulting antioxidant activity of sorbitol in bulk fish oils (**Table 3**). Again, equilibration conditions that favored more extensive dispersibility of polyols into the fish oil, i.e., longer times at an elevated temperature (90 °C), yielded greater antioxidant activities in the bulk fish oil samples than for shorter treatments. Furthermore, the greater suppression of peroxide values was also accompanied by less extensive or severe oxidized fish oil flavor development (**Table 3**).

Interpretation of these data for polyol antioxidant activity and dispersibility is hindered because conventional wisdom does not readily accommodate a concept where the polar polyols would be sufficiently dispersible or soluble in triacylglycerols to account for the antioxidant activity observed. Furthermore, references to solubility of polyols in triacylglycerols in the literature could not be found. Therefore, attempts were made to measure concentrations of dispersed sorbitol in bulk fish oil (excess, 2 h, 90−110 °C, 1 mmHg) using ^{1}H NMR and FT-IR analysis. Results for FT-IR analyses are shown in **Table 4**. Only very slightly greater OH stretch peak (band) areas were obtained for treated oils as compared to the control oil, thus preventing an unequivocal demonstration of dispersed sorbitol in fish oil by FT-IR. Sorbitol was not detected by ^{1}H NMR (data not shown) because the dispersed concentration of sorbitol was below the detection limit of the ^{1}H NMR instrumentation. During sampling, attempts were made to include a high proportion of the air−oil interface location into samples for instrumental analysis, but this effort did not yield measurable concentrations of polyols. Gravimetric measurements to determine the extent of sorbitol dispersion into fish oil were likewise unsuccessful.

In a subsequent series of trials to determine an estimate of the lower detection limit of sorbitol by FT-IR, various concentrations of sorbitol were dissolved in pyridine for analysis. The OH stretch band (3200−3600 cm^{-1}) for sorbitol was marginally detectable at a concentration of 1000 mg/kg of sorbitol in

Table 3. Effect of Time of Equilibration of Excess Sorbitol (10% by Weight) with Deodorized Fish Oil at 90 °C and 1 mmHg upon the Oxidative Stability of Fish Oils Subsequently Held under Light (2550 Lx) for 24 h at 5 °C

	antioxidant activity			
bulk fish oil treatment	time of equilibration of sorbitol at with fish oil (min)	peroxide value (μM hydroperoxide per mg fish oil)	change relative to control (%)	flavor description for fish oil
control (no sorbitol exposure)		5.5[a]	*b*	fishy green, burnt
equilibrated with sorbitol (10% by weight) at 90 °C	1	5.5	0	fishy green, burnt
	10	5.3	4 (–)[c]	fishy green, burnt
	30	4.9	12 (–)	fishy green, burnt
	60	3.4	39 (–)	green, slightly fishy
	120	2.9	46 (–)	green

[a] Average of four replicate analyses. Standard deviation <±0.1 for all determinations. [b] Not applicable; sorbitol was not added to control samples. [c] Extent and direction of change.

Table 4. Influence of Equlibration (2 h, 90–110 °C, 1 mmHg) of Selected Sugars and Polyhydric Alcohols (10% by Weight, Excess) with Deodorized Fish Oil upon the Area of the OH Stretch Band from FT-IR Analyses of Polyol-Treated Fish Oil Samples

fish oil treatment	area of OH stretch band[a]	changes relative to control (%)
control	7.152	*b*
+ raffinose	7.190	0.5 (+)[c]
+ sucrose	7.196	0.6 (+)
+ sorbitol	7.271	1.6 (+)
+ fructose	7.295	2.0 (+)
+ mannitol	7.343	2.6 (+)

[a] Integrated areas (cm^2) for 3200–3600 (cm^{-1}) wavenumbers. [b] Control no polyol exposure. [c] Extent and direction of change.

pyridine (data not shown); thus, it was concluded that the amount of sorbitol dispersed in the fish oils was below this concentration. Because the instrumental analyses attempted were inadequate to measure the amount of dispersed sorbitol in fish oil, it is suggested that future efforts utilize a radioisotope approach to unambiguously quantify the concentration of polyols in triacylglycerols (e.g., fish oil).

Factors Influencing the Antioxidant Activity and Apparent Dispersibility of Sugars and Polyhydric Alcohols into Bulk Fish Oils. A substantial antioxidant activity was demonstrated for each of the selected sugars and polyhydric alcohols when dispersed at 105−110 °C for 2 h at 1 mmHg into bulk fish oils (**Table 5**). In fact, the antioxidant activity under light for some of the polyols as compared favorably with that of the widely used phenolic antioxidant, TBHQ (200 ppm; **Table 5**). However, further experiments to determine whether the polyols and phenolic antioxidants provide synergistic antioxidant activities in bulk fish oil were not carried out.

The antioxidant activity in the dark for selected polyols (fructose or sorbitol) in refined (nondeodorized) bulk fish oils also was assessed using accelerated oxidation conditions of 60 °C (**Table 6** and **Figure 1**). Refined fish oil was employed in this trial to provide a substantially higher level of hydroperoxides than those found in freshly deodorized fish oil where they have been largely thermally degraded and removed by the process. While both fructose and sorbitol rendered some antioxidant activity in the refined bulk fish oil under dark conditions, sorbitol exhibited a much greater effect than fructose (**Table 6**). The somewhat high temperature conditions (60 °C) employed for the evaluation of antioxidant activity in the dark were chosen to provide an enhanced rate of hydroperoxide disproportionation (see initial and 2 h data points in **Figure 1**), which are mediated primarily by reduced transition metals (copper and iron ions; *16*, *19*, *20*).

Table 5. Influence of Some Physical Properties of Sugars and Polyhydric Alcohols Dispersed in Fish Oil by Holding (Excess) for 2 h at 105–110 °C and 1 mmHg upon Their Antioxidant Efficacy in Bulk Fish Oils When Subsequently Held under Light (2550 Lx) for 24 h at 5 °C

	antioxidant activity		physical properties of polyols	
bulk fish oil treatments[a]	peroxide value (μM hydroperoxide per mg of fish oil)	change relative to control (%)	melting point (°C)[e]	molecular weight[e]
control (no polyol added)	5.6[b]	*c*		
+ fructose	2.9	48 (–)[d]	103–105	180.16
+ sorbitol	3.6	36 (–)	110–112	182.17
+ raffinose	4.1	27 (–)	118	504.46
+ mannitol	4.7	16 (–)	168	182.17
+ sucrose	3.8	32 (–)	185	342.30
comparison with phenolic antioxidant (TBHQ)				
control (no additive)	7.9			
+ TBHQ (200 ppm)	3.1	61 (–)		

[a] Deodorized fish oil equilibrated with excess (10% by weight) sugars or polyols. [b] Average of four replicates; standard deviation <±0.2 for all determinations. [c] Control; no polyol added; not applicable. [d] Extent and direction of change. [e] Cited from Merck Index (1989).

Table 6. Influence of Equlibration of Fructose or Sorbitol (Excess; 10% by Weight) with Deodorized Fish Oil (2 h, 90–110 °C, 1 mmHg) upon the Oxidative Stability of the Fish Oils Subsequently Held in the Dark for 24 h at 60 °C

	antioxidant activity	
bulk fish oil treatment	peroxide value (μM hydroperoxide per mg fish oil)	change relative to control (%)
control	13.7[a]	*b*
+ fructose	11.9	13 (–)[c]
+ sorbitol	5.9	56 (–)

[a] Average of four replicate analyses; standard deviation <±0.3 for all determinations. [b] Not applicable; polyols were not added to the control sample. [c] Extent and direction of change.

Refined fish oils have been reported to usually contain about 1.5 and 0.1 mg/kg of iron and copper, respectively (*21*). Karahadian and Lindsay (*14*) added 20 ppm cupric palmitate to cod liver oil. They observed that cupric ion (Cu^{2+}) greatly increased the concentrations of *Z*-4-heptenal and both decatrienal isomers (*E*,*E*,*Z*-2,4,7- and *E*,*Z*,*Z*-2,4,7-), which contribute very unpleasant fishy−burnt, cod liver oil-like flavors to oxidizing fish oils. Similar reductions in concentrations of these compounds were seen in this study for sorbitol and mannitol but not for fructose, sucrose, or raffinose treatments (**Table 7**). Thus, the greater antioxidant activity of sorbitol as compared to

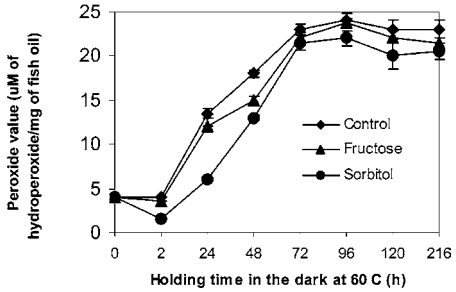

Figure 1. Antioxidant activity of dispersed fructose and sorbitol in refine undeodorized bulk fish oil (10% by weight, excess, 2 h at 105–110 °C, 1 mmHg) under accelerated oxidation (60 °C).

Table 7. Effect of Equilibration Introduction of Sugars and Polyhydric Alcohols (Excess; 10% by Weight; Held for 2 h at 1 mmHg and 105–110 °C) into Deodorized Fish Oils upon Concentrations of Volatile Compounds in Fish Oils after Subsequently Holding under Light (2550 Lx) for 24 h at 5 °C

	changes[a] in concentrations relative to control sample(%)				
volatile compounds	sorbitol	fructose	raffinose	sucrose	mannitol
hexanal	17 (–)	36 (+)	30 (+)	8 (+)	28 (–)
(*E*)-2-hexenal	24 (–)	11 (–)	2 (+)	28 (–)	29 (–)
(*Z*)-4- heptenal	38 (–)	2 (+)	6 (+)	29 (+)	59 (–)
(*Z*)-3-hexen-1-ol		12 (+)	15 (–)	18 (–)	79 (–)
2-octenal	9 (–)	36 (+)	32 (+)	27 (+)	16 (–)
(*E*,*Z*)-2,4-heptadienal	25 (–)	56 (–)	72 (–)	67 (–)	84 (–)
1,5-octadien-3-ol	33 (–)	16 (–)	44 (–)	51 (–)	63 (–)
(*E*,*E*)-2,4-heptadienal	27 (–)	36 (–)	52 (–)	56 (–)	50 (–)
(*E*,*Z*)-3,5-octadien-2-one	16 (–)	17 (–)	37 (–)	38 (–)	15 (–)
(*E*,*E*)-3,5-octadien-2-one	34 (–)	55 (–)	14 (–)	76 (–)	52 (–)
(*E*,*Z*)-2,6-nonadienal	42 (–)	7 (–)	5 (+)	8 (+)	2 (+)
(*E*,*Z*)-2,4-decadienal	6 (–)	58 (+)	17 (+)	27 (+)	18 (+)
(*E*,*E*)-2,4-decadienal	27 (–)	9 (–)	24 (–)	6 (–)	23 (+)
(*E*,*Z*,*Z*)-2,4,7-decatrienal	22 (–)	1 (+)	22 (+)	10 (–)	23 (–)
(*E*,*E*,*Z*)-2,4,7-decatrienal	33 (–)	19 (–)	4 (+)	15 (–)	19 (–)

[a] Extent (%) and direction of change relative to control, nontreated sample; (–) = decrease; (+) = increase.

fructose in bulk fish oil in the dark could be attributed to its ability to chelate transition metals (*22*, *23*) and thereby suppress fishy flavors (*14*). Nevertheless, on the basis of the antioxidant effects of sorbitol and fructose observed for these conditions, it is possible that polyols also function, to some extent, as free radical scavengers (**Figure 2**) in bulk fish oils in a manner similar to that described for other systems (*23*−*26*).

Because increasing the temperature and time of equilibration of bulk fish oil with polyols provided a pattern of increasing antioxidant activity, physical and chemical factors influencing dispersions that were in concert with these conditions and observations were further evaluated. It had been anticipated that polyols with lower melting points would more favorably disperse in bulk fish oils than those with higher melting points. Although the data in **Table 5** reveal that lower melting points sugars and polyhydric alcohols (fructose and sorbitol) generally yielded higher antioxidant activities, a clear association between melting point and antioxidant activity was not established.

Studies by Ueda and Matsumoto (*27*) on the effects of sugars on the physicochemical properties of water/oil (paraffin)/water (W/O/W) emulsions appear to provide potential seminal insights into the phenomenon of dispersion of sugars and polyhydric alcohols into nonpolar oils. These workers found that some sugars readily permeated the paraffin oil layer of W/O/W emulsions when they were placed in the inner water phase at sufficiently high amounts to provide a concentration differential between the two water phases. Thus, the data of Ueda and Matsumoto (*27*) strongly suggested that bulk fish oil oils (triacylglycerols) similarly could be expected to accommodate some degree of dispersed polyol molecules within the triacylglycerol matrix. Such a dispersion would then provide polar chelating and free radical-scavenging molecules both at the air−oil interface and within bulk triacylglycerol matrices.

Ueda and Matsumoto (*27*) further discovered that the ability of sugars to permeate the paraffin oil layer of W/O/W emulsions was governed by both the molecular weight and the average number of equatorial OH (e-OH) groups presented in sugars. Both lower molecular weights and lower numbers of e-OH groups for sugars were associated with higher permeation rates of sugars across the oil layer. Therefore, these parameters could be highly influential in governing the dispersibility of sugars into bulk fish oils.

The data in **Table 8** are in complete agreement with a concept that lower molecular weight sugars with a lower number of e-OH groups also migrate more efficiently into fish oil matrices than those with a higher number of e-OH groups and larger molecular weights. As a result, sugars that have a high number of e-OH groups and a high molecular weight provide less efficient antioxidant activity in bulk fish oil because they are less likely to permeate in the triacylglyceral matrix. Overall, the data suggest that the bulk fish oil matrix provides suitable host sites for polyols, but the physical distribution of sugars and polyhydric alcohols within bulk fish oil matrices or even whether they substantially remain in bulk fish oils after treatment is undetermined. Polyols may be concentrated at the air−water interface, but considering the method of indexing antioxidant activity used (peroxide value) and the dramatic increases in antioxidant activity caused by increased times and temperatures of fish oil−polyol equilibrations (**Tables 2** and **3**), it seems that polyol distribution and antioxidant activity within the bulk matrix of fish oil are also possible.

Because only sugars exhibit axial and equatorial positioning of OH groups on a ring, these stereochemical features would not apply to polyhydric alcohols. However, other stereochemical features relating to the vicinal positioning of OH groups on the linear carbon backbone of polyhydric alcohols may also be influential (e.g., sorbitol vs mannitol), particularly in relation to their abilities to permeate fish oils and chelate transition metals.

Performance of Polyol-Stabilized Bulk Fish Oils in Emulsions. Bulk fish oils that had been equilibrated with sugars and polyhydric alcohols (105−110 °C for 2 h at 1 mmHg) were each prepared into model fish oil emulsions. The formula of the emulsion had been established in earlier studies on the antioxidant activity of polyols introduced only into the aqueous phase at the time of preparation. The data in **Table 9** show greatly suppressed antioxidant activity for polyol-stabilized bulk fish oils when incorporated into emulsions as compared to their activity in bulk fish oils (**Table 5**). Furthermore, the antioxidant activities of sugars and polyhydric alcohols were similar in emulsions regardless of whether the polyols were introduced as an ingredient in bulk fish oil or added as a direct ingredient into the aqueous phase (16% of the aqueous phase) of the emulsions.

The composite emulsion data strongly indicate that fish oil dispersed polyols are somewhat loosely associated with bulk fish oil, which permits their redistribution into the aqueous phase, the oil−water interface, and perhaps the bulk oil matrix when the fish oils are prepared into an emulsion. Similarly, polyols introduced solely into the water phase apparently also

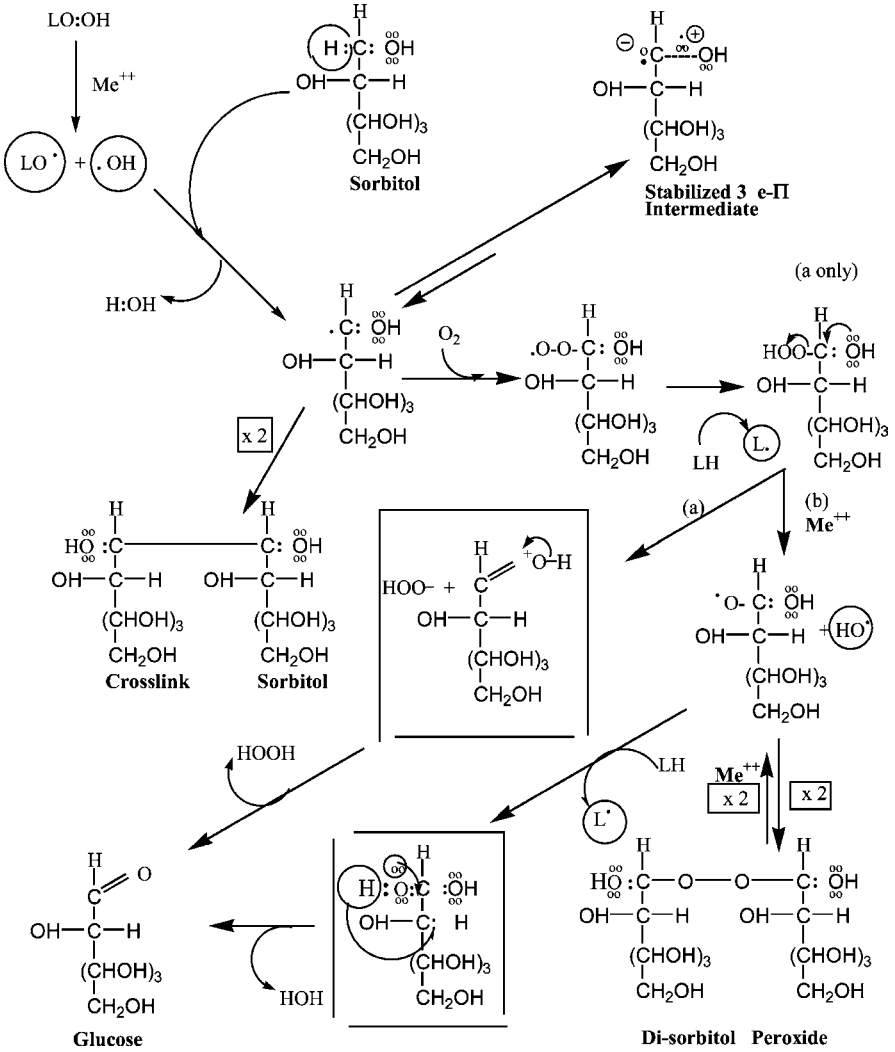

Figure 2. Some proposed mechanistic schematic compounds from free radical (•OH) scavenging by sorbitol and other polyols in bulk fish oils, including the role for the stabilized (3 e-π) intermediate from the polyol (*28*).

Table 8. Relationship between Sugar Mobility Across Paraffin Oil Layers in W/O/W Emulsions and Apparent Dispersibility and Antioxidant of Sugars in Bulk Deodorized Fish Oils

	antioxidant activity		physical properties of polyols		
bulk fish oil treatment[a]	peroxide value (μM hydroperoxide per mg of fish oil)	change relative to control (%)	average number of equatorial OH sugars	approximate velocity of sugar flux (μM/g sample/h) in W/O/W emulsions	molecular weights[h]
control	5.6[b]	*c*			
+ fructose	2.9	48 (–)[d]	3.0[e]	0.15[e]	180.16
control	5.6				
+ sucrose	3.8	32 (–)	6.3[e]	0.05[e]	342.30
control	5.6				
+ raffinose	4.1	27 (–)	8.3[f]	NA[g]	504.46

[a] Deodorized fish oil equilibrated with excess (10% by weight) sugars or polyols at 105–110 °C for 2 h at 1 mmHg and then held for 24 h under light (2550 Lx) at 5 °C. [b] Average of four replicates; standard deviation <±0.2 for all determinations. [c] Control; no polyol added. [d] Extent and direction of change. [e] Cited from ref *27*. [f] Cited from Uedaira et al. (1989). [g] Not available. [h] Cited from Merck Index (1989).

readily distribute into various locations of emulsions to provide an equilibrium situation whose antioxidant activity performs very similar to that seen for the polyol-equilibrated fish oils prepared into emulsions.

Although these studies showed that treatment of bulk fish oils with polyols provided some protection to the oils, incorporation of polyol-containing fish oils into emulsions greatly reduced the antioxidant efficacy to a level similar to that obtained by simply introducing polyols (16%) into the aqueous phase. Nevertheless, use of the polyol-treated bulk fish oils in emulsions appears to provide a means to overcome potentially excessive sweetness provided by the use of 16% solutions of sugars and polyols in the aqueous phase of emulsions because only low concentrations of polyols (<1000 mg/kg, estimated) remain in bulk fish oils after direct equilibration of polyol treatments at elevated temperatures (excess polyol; 90−110 °C).

Introduction of Sugars and Polyhydric Alcohols into Bulk Fish Oils via Propylene Glycol Solutions. Because effective levels of sugars and polyhydric alcohols introduced by equilibration of an excess amount of polyol at 90−110 °C with fish

Table 9. Influence of Method of Introduction of Sugars and Polyols upon the Oxidative Quality of Fish Oil Emulsions Held under Light (2550 Lx) for 24 h at 5 °C

	antioxidant activity in emulsions			
	polyols introduced into bulk fish oil[a]		polyols introduced into the aqueous phase (16%)[b]	
emulsion descriptions	peroxide value (μM hydroperoxide per mg fish oil)	change relative to control (%)	peroxide value (μM hydroperoxide per mg fish oil)	change relative to control (%)
control (no polyols)	8.5[c]	*d*	4.0	
+fructose	7.2	16 (–)	3.3	18 (–)
control	12.0		4.0	
+ sorbitol	10.1	18 (–)	3.3	18 (–)
control	12.0		6.6	
+ raffinose	10.3	15 (–)	5.6	15 (–)
control	12.0		4.0	
+ sucrose	10.3	14 (–)	3.1	23 (–)
control	12.0		6.6	
+ mannitol	9.3	23 (–)	5.9	11 (–)

[a] Composition: 70 mL of fish oil, 26.8 mL of water, and 3.2 g of Tween 80. The excess polyol (10% by weight) was equilibrated with fish oil at 105–110 °C for 2 h at 1 mmHg. [b] Composition: 70 mL of fish oil, 22.5 mL of water, 4.32 g of polyol, and 3.2 g of Tween 80; results not shown. [c] Average of four replicate analyses. Standard deviation <±0.6 for all determinations. [d] Extent and direction of change.

Table 10. Influence of Time and Tempreture of Dispersion of Propylene Glycol (0.3%) into Deodorized Fish Oil upon the Oxidative Quality of the Oil after Exposure to Light (2550 Lx) for 24 h at 5 °C

	antioxidant activity		
bulk fish oil treatment	peroxide value (μM hydroperoxide per mg fish oil)	change relative to control (%)	flavor assessment of fish oil
	21 °C set (time = 1 h)		
control	7.8[a]	*b*	fishy, green
+ propylene glycol (0.3%)	6.8	12 (–)[c]	green
	90 °C set (time = 0.25 h)		
control	10.8		fishy, burnt
+ propylene glycol (0.3%)	9.0	14 (–)	very green, fishy

[a] Average of four replicate analyses. Standard deviation <±0.8 for all determinations. [b] Control; no polyol was added to control samples. [c] Extent and direction of change.

oil required a lengthy holding period (2 h), means to shorten this time were explored. Both glycerol and propylene glycol were considered as solvents for polyols because of their polarity and fluid properties at room temperature (Merck Index, 1989). However, propylene glycol was chosen for evaluation because it was more compatible with the polarity of fish oils; thus, solutions were more readily prepared.

Propylene glycol was visually completely dissolved in fish oil up to a maximum level of about 0.3%. The data in **Table 10** indicate that 0.3% propylene glycol was dispersed into bulk fish oil equally well at both 21 and 90 °C when stirred for 1 h before use in antioxidant efficacy testing. The data in **Table 10** further revealed that the introduction of 0.3% propylene glycol into deodorized fish oil, and subsequently holding for 24 h under light (2550 Lx) at 1 mmHg, resulted in only modest antioxidant activities (−12 to −14% reductions in peroxide values). Furthermore, the conditions of introduction (21 °C for 1 h or 90 °C for 0.25 h) in fish oil did not appear to greatly affect the antioxidant activity provided by 0.3% propylene glycol in fish oils held under light-accelerated oxidation conditions. On the basis of earlier trials, the relatively low antioxidant activity provided by 0.3% propylene glycol in fish oil seemed to be provided mainly by free radical scavenging rather than by transition state metal chelation.

Fructose and sorbitol (25% by weight) were readily soluble in propylene glycol with heating and stirring at 90 °C. Introduction of either fructose or sorbitol in propylene glycol solutions, up to 0.1% of polyol plus 0.3% propylene glycol, yielded complete mutual solubility of the mixtures as determined by visual inspection. In preliminary antioxidant efficacy testing of various concentrations of polyols delivered in propylene glycol to fish oils, it was found that fructose or sorbitol used at 0.025 or 0.05% in fish oils gave better or equivalent antioxidant activities than either 0.01 or 0.10% levels (data not shown). Thus, data for the introduction of 0.025 and 0.05% levels of polyols into fish oils via propylene glycol solutions were selected to illustrate the antioxidant activities of polyols introduced via propylene glycol solutions (**Table 11**).

At 21 °C, only slightly greater degrees of antioxidant activity were observed for treatments involving introduction of fructose and sorbitol in propylene glycol into fish oil (**Table 11**) as compared to that seen when propylene glycol was used alone (**Table 10**). The slight elevations in antioxidant activities seen

Table 11. Influence of Equlibration Temperature and Amount of Fructose and Sorbitol Dissolved in Propylene Glycol Introduced into Deodorized Fish Oil upon with Oxidative Quality of the Oil after Exposure to Light (2550 Lx) for 24 h at 5 °C

	antioxidant activity		
bulk fish oil treatment	peroxide value (μM hydroperoxide per mg fish oil)	change relative to control (%)	flavor assessment of fish oil
	21 °C set (equilibration time = 1 h)		
control	7.8[a]	*b*	fishy, green
+ sorbitol (0.025%) and propylene glycol (0.3%)	6.6	16 (–)[c]	green, nutty
+ sorbitol (0.05%) and propylene glycol (0.3%)	6.6	16 (–)	slightly fishy, green
control	6.3		fishy, green, burnt
+ fructose (0.025%) and propylene glycol (0.3%)	4.9	21 (–)	bland, green, nutty
+ fructose (0.05%) and propylene glycol (0.3%)	5.6	10 (–)	strong green
	90 °C set (equilibration time = 0.25 h)		
control	10.8		burnt, fishy
+ sorbitol (0.025%) and propylene glycol (0.3%)	6.9	36 (–)	fishy, green, slightly burnt
+ sorbitol (0.05%) and Propylene glycol (0.3%)	7.9	26 (–)	green, nutty
control	10.6		very fishy, green burnt
+ fructose (0.025%) and propylene glycol (0.3%)	6.6	38 (–)	bland, slightly green
+ fructose (0.05%) and propylene glycol (0.3%)	7.8	26 (–)	green, slightly fishy

[a] Average of four replicate analysis; Standard deviation < ± 0.8 for all determinations. [b] Control; No polyol added to control samples. [c] Extent and direction of change.

for sorbitol and fructose added in propylene glycol at 21 °C were roughly equivalent to those observed for the ambient temperature (21 °C) equilibration introduction of these polyols into fish oil (excess polyol; **Table 1**).

However, introduction of fructose and sorbitol via propylene glycol solution into fish oil at 90 °C (0.25 h) resulted in elevation of antioxidant activities (**Table 11**) to levels approaching those seen for elevated temperature (90−115 °C) equilibration introductions of polyols into fish oils (**Table 5**). Because the introduction of fructose and sorbitol into fish oil via propylene glycol solutions at 21 °C visually appeared to result in complete physical dispersion or solution, an explanation for enhanced antioxidant activity that was induced by heating (90 °C) during introduction of polyols in propylene glycol was not readily discernible. However, it is thought that heating increases molecular mobility within the fish oil matrix, which in turn enhances contact of polyols with transition metals and promotes hydroperoxide decomposition with subsequent polyol radical scavenging reactions.

Interestingly, there was no evidence for additive or synergistic antioxidant activities between propylene glycol and dissolved polyols (fructose, sorbitol; **Tables 5**, **10**, and **11**). In fact, when data for incorporation of fructose or sorbitol at 0.05% in propylene glycol are considered, notably lower antioxidant activities are observed (approximately 10%) as compared to fish oils containing only 0.025% fructose or sorbitol in propylene glycol. Thus, unfavorable competitive chemical or physical interactions or displacements in the bulk fish oil occur and these interfere with one or more of the various antioxidant mechanisms contributed by higher molecular weight polyols (sorbitol and fructose).

Influence of Dispersed Polyols on Flavor Quality of Bulk Fish Oils. In addition to suppressing oxidation rates of fish oils, introduction of sugars and polyhydric alcohols into bulk fish oils also improved the flavor quality of fish oils as compared to untreated controls (**Tables 3** and **11**). The polyol treatments that resulted in large reduction in peroxide values also reduced the content of selected volatile oxidation products in fish oils (**Table 7**). Thus, the formation of key oxidized flavor compounds, including hexenal, (*Z*)-4-heptenal, and the (*E*,*Z*)- and (*E*,*E*)-2,4-heptadienals was reduced. Sorbitol and mannitol, both effective transition metal chelators (Dutton et al., 1948; Cowen et al., 1962), also greatly reduced the concentrations of fishy−burnt-flavored (*E*,*Z*,*Z*)- and (*E*,*E*,*Z*)-2,4,7-decatrienals.

In conclusion, polyols were found to serve as antioxidants in fish oils as reflected in peroxide values of samples upon exposure to fluorescent lighting. By increasing the time and temperature of equilibration, the antioxidant activity of polyols was enhanced greatly. However, the content of dispersed polyols in bulk fish oil was below the detection limits of both ^{1}H NMR FT-IR techniques employed.

Exposure of fish oils containing polyols to accelerated oxidation conditions revealed that sorbitol provided antioxidant activity mainly by chelating transition metals, but perhaps free radical scavenging was also involved, possibly attributable to hydroxyl radical scavenging activity in the case of fructose.

Lower molecular weights and smaller numbers of e-OH groups for sugars were associated with higher rates of permeation of sugars into fish oil and, hence, greater antioxidant activity as compared to higher molecular weights and larger numbers of e-OH groups.

Incorporation of polyol-containing fish oils into emulsions greatly reduced the antioxidant activity of polyols as compared to bulk fish oils. The presence of polyols in propylene glycol added to bulk fish oil showed antioxidant activities of the same magnitude as when polyols were introduced by equilibration. Polyols in bulk fish oils suppressed both oxidation and formation of volatile off-flavors, including (*Z*)-4-heptenal, (*E*,*E*)- and (*E*,*Z*)-2,4-heptadienals, and (*E*,*Z*,*Z*)- and (*E*,*E*,*Z*)-2,4,7-decatrienals. However, the mechanisms involved remain unclear. Furthermore, synergism between polyols and phenolic antioxidants in fish oils needs to be investigated. It is also necessary to employ a radioisotope approach to quantify the amount of polyols present in fish oils after treatments.

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