Characterization of the Antioxidant Activity of Sugars and Polyhydric Alcohols in Fish Oil Emulsions

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Polyols have been incorporated into fish oil emulsions as a means for the inhibition of lipid oxidation and suppression of fishy flavor. However, the role of sugars and polyhydric alcohols as antioxidants has not been clearly established. Selected polyols were evaluated for their performance as antioxidants and modifiers of oxidation pathways in a model system. Oil/water (O/W) emulsions were prepared with freshly steam-deodorized menhaden oil. A layer of emulsion in aluminum pans held at 5 °C was exposed to 2550 lx fluorescent lights for 24 h before peroxide values and volatile flavor compounds were analyzed by GC headspace entrainment procedure. Antioxidant activity was confirmed for fructose, sucrose, raffinose, sorbitol, or mannitol when incorporated at 16% of the aqueous phase into model fish oil-in-water emulsions. Peroxide values were suppressed 10−18% in treated samples compared to control samples. Viscosity data did not exclude possible contributions from a restricted oxygen diffusion mechanism in the antioxidant activity, but revealed that emulsion viscosity did not govern fish oil oxidation rates. Combining polyols with phenolic antioxidants (α-tocopherol, BHT, or TBHQ) frequently diminished the antioxidant activity compared to that for individual phenolic antioxidants, which was interpreted as indicating that the H-donating activity of phenolic antioxidants was hindered by the H-bonding activity of polyols. A viscosity-based inhibition of the retroaldol conversion of (*E,Z*)-2,6-nonadienal to (*Z*)-4-heptenal with a high fructose concentration (67%) was attributed to a restriction of molecular mobility of reactants, but the conversion was only slightly inhibited by the concentration of fructose (16%) used in experimental emulsions. The data supported a hypothesis that either or both free radical scavenging and transition state metal chelation activities were provided by polyols in fish oil emulsions. Also, polyols retarded the water-requiring retroaldol decomposition of (*E,Z*)-2,6-nonadienal to (*Z*)-4-heptenal in the model systems and the reaction may be involved in some suppression of fishy flavors in emulsions.

KEYWORDS: Sugars; polyhydric alcohols; antioxidant activity; fish oils; emulsions; lipid oxidation; volatile compounds

INTRODUCTION

Aldehydes derived from the autoxidation of long-chain polyunsaturated fatty acids contribute pronounced, unpleasant flavors to bulk fish oils and foods containing fish oil ingredients (*1−3*). Among the strategies employed to suppress fishy flavors, oxygen-exclusion packaging (*4*) and oxygen-barrier encapsulation of fish oils (*5−7*) have afforded some control of off-flavor development in commercial settings.

However, the introduction of antioxidants remains a major strategy for suppressing oxidation of bulk fish oils and foods emulsions containing fish oils (*8−11*). Generally, typical antioxidant mixtures for fish oil stabilization incorporate a primary antioxidant [such as α-tocopherol or *tert*-butylhydroquinone (TBHQ)], possibly a supporting antioxidant (such as

ascorbyl palmitate), and a chelating agent (such as citric acid), and various degrees of success have been claimed for these mixtures (*9, 11, 12*). However, because reliable suppression of unpleasant, fishy flavors in either oils or foods containing substantial long-chain polyunsaturated fatty acids is not easily achieved with current antioxidant mixtures, means for providing additional antioxidant protection and fishy flavor suppression would be highly beneficial.

Incorporations of sugars and polyhydric alcohols into fish oil emulsions have been patented by several researchers as novel means for the suppression of both fishy flavor development and lipid oxidation in fish oil emulsions (*13−15*). Research has generally shown that sugars and polyhydric alcohols provide antioxidant activity in various systems (*16−19*), but some have reported that reducing sugars may act as prooxidants in emulsions (*20−23*).

The mechanisms by which sugars and polyhydric alcohols exert their antioxidant effects have not been clearly established (*18*). Initially proposed mechanisms invoked chelation of

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prooxidant metals by sugars and polyhydric alcohols (*24, 25*), and others have supported this view (*23, 26, 27*). However, Sims et al. (*28*) have argued that sugars and sugar alcohols suppress oxidation of lipids in emulsions by viscosity restriction of oxygen diffusion in the disperse aqueous phase (*29*) and through oil–water interfaces (*30*) rather than by intervention through a chemical mechanism. Others (*31–34*) likewise have supported the view that sugars and polyhydric alcohols provide at least some antioxidant functionality in emulsions through matrix or viscosity-regulated oxygen diffusion mechanisms.

Ponginebbi et al. (*18*) found that viscosity did not account for all of the antioxidant protective effects of higher concentrations of sucrose in linoleic acid emulsions and concluded that mechanisms other than viscosity also were involved. Antioxidant activity via free radical scavenging, especially for the hydroxyl radical (•OH), by sugars and polyhydric alcohols in aqueous systems is a well-accepted phenomenon (*17, 19, 35, 36*). Furthermore, support has been building for the view that sugars function as free radical scavengers in the control of free radical oxidation in biological systems (*17, 26, 37*). However, free radical scavenging by sugars has not been discussed to any extent in relation to the oxidative stability of fish oil emulsions.

Because sugars and other polyols suppress oxidation-derived fishy flavors in fish oil emulsions, a further understanding of the mechanism of this activity should provide insights into formulations for more effective antioxidant stabilization of fish oils in emulsified foods. Therefore, the purpose of this study was to characterize the mechanisms involved in the suppression of fishy flavors and lipid oxidation in fish oil emulsions by sugars and polyhydric alcohols and to develop further information about the use of sugars and polyhydric alcohols as antioxidants in fish oil emulsions.

MATERIALS AND METHODS

Materials. Commercial (RBU-D code 548, SPMO brand non-deodorized menhaden oil, refined, bleached, and undeodorized dietary grade; Omega Protein, Reedville, VA) was obtained and stored in the dark in a closed container at 5 °C until used (<6 months). Sorbitol, fructose, raffinose, mannitol, lactose, sucrose, and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). Butylated hydroxytoluene (BHT) and TBHQ were donated by Eastman Chemical (Kingsport, TN). All other chemicals were of reagent or more refined grades.

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 (*38, 39*). The initial peroxide value of menhaden oil after deodorization was 0.02 ± 0.001 µM hydroperoxide/mg of fish oil.

Preparation of Fish Oil Emulsions. Fish oil emulsions were generally modeled after those described by Antrim and Tylor (*15*) and Hsieh and Regenstein (*40*). 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 emulsification was initiated. α-Tocopherol, BH%, and TBHQ were dissolved in fish oil and then introduced into emulsions.

Conditions for Accelerated Oxidation and Method for Indexing Oxidation in Samples. Fish oil emulsions were exposed to lighted conditions (fluorescent, daylight-type; ~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. Progress of oxidation was monitored by determining peroxide values using the procedure described by Buege and Aust (*41*). Standard deviations of means were also calculated for these data.

Aroma and Flavor Assessments of Fish Oil Emulsions. Fish oil 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 Fish Oil Emulsion Samples. Volatile compounds in fish oil emulsions were quantitatively measured using the dynamic headspace procedure described by Olafsdottir et al. (*42*).

Headspace volatiles in fish oil 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 ~1 mL of redistilled diethyl ether (Mallinckrodt, Paris, KY) into a Concentrate tube (Laboratory Research Co., Lonita, CA). Each isolate was then concentrated for GC analysis under a slow nitrogen stream to ~10 µL at ambient temperature.

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

Coincidence of retention indices ($I_{E;[43]}$) for unknown peaks with those of volatile compounds identified in cod liver oil by Karahadian and Lindsay (*2*) were used for assignment of identities of compounds.

Percent changes of volatile compounds in treated samples compared to appropriate control (no polyols or phenolic antioxidants) were calculated from concentrations measured in the samples. When a volatile compound in the treated sample decreased in concentration compared to the control, the following formula was used:

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

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

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

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

Influence of Fructose on the Rate of (*E,Z*)-2,6-Nonadienal Retroaldol Conversion in Aqueous Solutions. The general model system for aldehyde retroaldol conversion described by Josephson and Lindsay (*44*) was employed. Similar batches were also prepared for a lesser concentrated aqueous fructose solution [16% (*14*)], but the pH was adjusted to pH 6.5, and the mixtures were stirred and covered with foil at ambient temperature (21 °C). Samples were withdrawn for analysis periodically over 120 h. The percent conversion of (*E,Z*)-2,6-nonadienal via retroaldol condensation was based on measured concentrations of (*Z*)-4-heptenal resulting from the reaction.

Methods for Physical and Microbiological Characterization of Fish Oil Emulsions. Emulsion samples were treated according to AOAC (*45*) recommendations (preparation, calibration, and determination) for water activity measurements of emulsions. Aerobic plate counts for selected emulsions were performed using the procedure described in the *Compendium of Methods for the Microbiological Examination of Foods* (*46*).

RESULTS AND DISCUSSION

Influence of Polyols on the Oxidation of Fish Oil in Emulsions Held under Light for 24 h at 5 °C. Results of

Table 1. Influence of Selected Sugars and Polyhydric Alcohols (4.32% of Formula; 16% in Aqueous Phase) upon the Oxidative Quality of Model Fish Oil (70%) Emulsions Held under Light (2550 lx) for 24 h at 5 °C

emulsion treatment	peroxide value		headspace odor description	viscosity (cpi)		solubility in water (g/100 at 20 °C)
	μM hydroperoxide/ mg of fish oil	change relative to control (%)		initial (21°C)	after holding (24 h, 5°C)	
control	4.0[a]	–[b]	oxidized–painty	8750[d]	13750	–
+ fructose	3.3	18 (–)[c]	oxidized–painty	18750	18750	78[e]
control	4.0	–	oxidized–painty	–	–	–
+ sorbitol	3.3	18 (–)	oxidized–painty	16250	21500	72[e]
control	4.0	–	oxidized–painty	–	–	–
+ sucrose	3.1	23 (–)	green–fishy	24375	25000	64[e]
control	6.6	–	oxidized–painty	–	–	–
+ raffinose	5.6	15 (–)	green–fishy	18125	26875	14.3[f]
control	6.6	–	oxidized–painty	–	–	–
+ mannitol	5.9	11 (–)	green–oxidized	39250	37500	14.7[g]

[a] Average of four replicate analyses: standard deviation < ±0.3 for all determinations. [b] Not applicable; no polyol added to control samples. [c] Extent and direction of change. [d] Spindle 6; rpm 2; centipoise; average of two replicate analyses; standard deviation < ±1900 for all determinations; one control sample for all viscosity comparisons. [e] Reference *54.* [f] Reference *56.* [g] Reference *55.*

oxidative quality assessments for selected fish oil emulsions containing sugars or polyhydric alcohols after exposure to fluorescent lighting (2550 lx) for 24 h at 5 °C are summarized in **Table 1**. In a series of preliminary trials (data not shown), it was found that the susceptibility to oxidation for a given lot of deodorized fish oil was governed by uncontrollable factors, but samples within a lot of fish oil behaved similarly. Therefore, because the number of fish oil emulsions (100 mL each) that could be prepared from a single deodorization run was limited, experiments were structured so that individual polyol treatments were compared to appropriate control emulsions prepared from the same lot of fish oil. Therefore, data are presented for both absolute measurements and percent change caused by a treatment compared to that for a control sample.

The concentration of polyol solute in the model emulsions employed (**Table 1**; 4.32% of the total, 16% of the aqueous phase) was based on the functionally effective level of fructose claimed in the patent issued to Scheroder and Muffet (*14*). The data in **Table 1** show that each of the polyols distinctly reduced the peroxide value (−11 to −18%) compared to its corresponding control sample prepared without added polyols, and these results were in general agreement with those reported by others (*14, 18, 28*).

Several research groups (*18, 28, 30−32*) have supported the view that elevated viscosities of the continuous aqueous phases of emulsions containing dissolved polyols inhibit oxygen diffusion and thereby cause a suppression of the oxidation of disperse phase lipids. The viscosity data also shown in **Table 1** for fish oil emulsions prepared with the various polyols reveal that the inclusion of each of the polyols at 16% of the aqueous phase substantially elevated the viscosity of the freshly prepared samples compared to the control sample (8750 cpi; at 21 °C). Because the model fish emulsions were held at 5 °C for 24 h under light for an oxidation challenge, viscosities were also measured after this exposure. Notable increases in viscosity were observed after holding at 5 °C for the control (+5000 cpi), sorbitol (+5250 cpi), and raffinose (+8750 cpi) treatments, whereas the reduced temperature exposure had little or no effect on the emulsions containing either fructose, sucrose, or mannitol.

Although each polyol was incorporated into the model emulsion at an equivalent level (16% of the aqueous phase), the resulting viscosities of emulsions containing polyols varied widely (16250−39250 cpi) at both 5 and 21 °C. Furthermore, the highest viscosities were observed for the mannitol (37500 cpi at 5 °C) and raffinose (26875 cpi at 5 °C) emulsions, and

these treatments resulted in the lowest absolute reductions in peroxide values (−11 and −15%, respectively) compared to control samples. When the water solubilities of the polyols were considered (**Table 1**), however, it appeared that higher degrees of water solubility were associated with lower viscosities and more effective reductions in peroxide values (fructose, sucrose, and sorbitol; −18 to −23%).

It is unlikely that saturation concentrations of fructose, sucrose, and sorbitol were achieved in aqueous phases of these emulsions even under the 5 °C experimental conditions, and thus they remained available in a solublized form in the emulsion systems for either or both transition state metal chelation or free radical-scavenging antioxidant activities. However, the poorly soluble polyols (mannitol and raffinose) exceeded saturation concentrations (∼14%) in the aqueous phases even at the time of preparation of model emulsions (added at 16% of the aqueous phase; ∼21 °C). As result, it would be expected that the insoluble, crystalline polyol at either 21 or 5 °C would not be available for chelation or radical-scavenging antioxidant activities and, thereby, could account for some of the diminished antioxidant activity seen for these polyols compared to the more soluble polyols (fructose, sucrose, and raffinose).

Because the incorporation of the polyols in each case notably increased the viscosity of resulting emulsions as well as lowered the peroxide value compared to control samples, the viscosity-based oxygen diffusion restriction mechanism of antioxidancy by polyols in fish oil emulsions cannot be dismissed entirely. However, the failure of a high emulsion viscosity to yield a high degree of antioxidancy (e.g., mannitol) indicated that this mechanism may be of limited importance in polyol antioxidant functionality in emulsions. On the other hand, the evidence favors chelation or substantial free radical-scavenging antioxidant contributions by polyols in fish oil emulsions because the most soluble polyols (sucrose, fructose, and sorbitol) showed the greatest antioxidant activity. Presumably, the dissolved polyols exerted most of their antioxidant activity at or near the water−oil interface (*47*), and the polar nature of polyols should favor their association with either metal ions or •OH radicals for effective scavenging of these species.

Influence of Polyols on the Aroma of Fish Oil Emulsions Held under Light for 24 h. Initially, all emulsions were free of aromas because they were prepared from odorless, freshly deodorized fish oils. However, as shown in **Table 1**, the conditions of exposure (2550 lx for 24 h) consistently yielded oxidized painty comments for control emulsions, whereas

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Table 2. Effect of Selected Sugars and Polyhydric Alcohols (4.23% of Formula; 16% in Aqueous Phase) upon the Concentration (Parts per Billion) and Relative Presence of Volatile Compounds in Fish Oil Emulsions Compared to a Nontreated Control Emulsion after Exposure to Light (2550 lx) for 24 h at 5 °C

| | polyol compounds in fish oil emulsions | | | | | | | | | |
| | fructose | | sorbitol | | sucrose | | raffinose | | mannitol | |
volatile compound	ppb[a]	% change[b]	ppb	% change	ppb	% change	ppb	% change	ppb	% change
hexanal	1672[c]	56 (−)	286	10 (−)	168	35 (−)	214	42 (+)	208	19 (−)
(*E*)-2-hexenal	874	34 (−)	34	3 (−)	15	57 (−)	26	65 (+)	9	74 (−)
(*Z*)-4- heptenal	1104	28 (−)	107	12 (−)	99	16 (−)	119	12 (+)	58	45 (−)
(*Z*)-3-hexen-1-ol	796	8 (−)	112	77 (+)	10	60 (−)	102	75 (+)	271	61 (+)
2-octenal	681	71 (−)	1081	21 (−)	873	17 (−)	974	8 (+)	591	44 (−)
(*E,Z*)-2,4-heptadienal	88	96 (−)	36	56 (+)	17	6 (+)	15	42 (+)	24	33 (+)
1,5-octadien-3-ol	184	90 (−)	13	81 (−)	80	14 (+)	16	71 (−)	56	19 (−)
(*E,E*)-2,4-heptadienal	711	25 (−)	14	30 (−)	19	5 (−)	16	16 (+)	17	14 (−)
(*E,Z*)-3,5-octadien-2-one	141	98 (−)	35	36 (−)	87	37 (+)	8	53 (+)	24	56 (−)
(*E,E*)-3,5-octadien-2-one	90	48 (−)	17	78 (−)	92	16 (+)	15	61 (+)	22	74 (−)
(*E,Z*)-2,6-nonadienal	388	6 (+)	8	88 (−)	217	70 (+)	4	3 (−)	24	26 (+)
(*E,Z*)-2,4-decadienal	52	35 (+)	20	85 (−)	252	47 (+)	26	45 (−)	20	13 (+)
(*E,E*)-2,4-decadienal	102	32 (−)	5	91 (−)	859	93 (+)	14	95 (−)	137	61 (+)
(*E,Z,Z*)-2,4,7-decatrienal	52	63 (−)	4	96 (−)	636	86 (+)	4	93 (−)	172	46 (+)
(*E,E,Z*)-2,4,7-decatrienal	48	36 (−)	3	98 (−)	186	16 (+)	3	96 (−)	30	81 (−)

[a] Concentration of volatile compound in treated emulsion. [b] Percent change relative to control, nontreated sample; (−) = decrease; (+) = increase. [c] Concentration of volatiles are within ±20% method variability.

emulsions prepared with sugars or polyhydric alcohols ranged from green−fishy to oxidized− painty. All of the aromas were assumed to be derived from chemical processes because microbial growth was not found during 14 days of incubation on nutrient agar (*46*).

Although flavor assessments by mouth provide a much more sensitive means for detection of off-flavors associated with oxidation of fish oils than assessments of corresponding aromas (*2*), taste interferences from the emulsifier employed in emulsion preparation precluded tasting the experimental emulsions. Instead, headspace aromas above emulsions were evaluated to provide an index for the sensory properties of the fish oil emulsions.

Fish oil quality should be assessed by a combination of sensory evaluation and headspace aroma. When assessed by mouth, the flavors of fish oils and foods containing fish oils progress consecutively during autoxidation through a range of qualitative descriptors according to the sequence of green-plant-like, fresh fish-like, burnt−fishy (trainy or cod liver oil-like), and finally oxidized−painty stages (*2*). The latter, more advanced flavor stages overlap in character because notable concentrations of both the (*E,E,Z*)- and (*E,Z,Z*)-2,4,7-decatrienals (burnt−fishy) and other lipid oxidation products associated with oxidized painty aromas [e.g., hexanal, (*Z*)-4-heptenal, and 2,4-heptadienals]. However, when fish oil products are assessed by aroma only, fishy-burnt aromas are subdued in part because of the low volatility of the 2,4,7-decatrienal isomers, and the burnt-fishy descriptor is not commonly invoked for aromas of systems exhibiting modest degrees of oxidation. Such was the case in this study, and although some emulsions containing polyols developed only to the stage of green−fishy aromas, others containing polyols and all control samples progressed to the oxidized−painty stage of aroma development. Presumably, these more advanced oxidation-stage aromas were also accompanied by burnt−fishy flavors.

Notably, emulsions prepared with either fructose or sorbitol yielded oxidized−painty aromas after the light exposure, although each also caused a reasonably large suppression of the peroxide value (−18%) compared to control samples (**Table 1**). Additionally, the absolute peroxide values for both the fructose and sorbitol emulsions were also quite low (3.3)

compared to those of some of the other polyol-treated samples (5.6 and 5.9; raffinose and mannitol, respectively), which exhibited less extensively oxidized flavors. Data from the quantitative measurement of selected volatile oxidation compounds in the polyol-treated sample are shown in **Table 2**, and it can be seen that some volatiles commonly associated with oxidized−painty flavors and aromas [hexanal, (*Z*)-4-heptenal, and (*E,Z*)- and (*E,E*)-2,4-heptadienals (*2*)] were elevated in the fructose- and sorbitol-treated emulsions. Overall, these data were in general agreement with the chemical and sensory oxidative assessments of the light-exposed fish oil emulsions. However, it appeared that the absolute quantitative measurement data of volatile compounds more accurately reflected assessed aromas than the degree of change (percent) observed between control emulsions and comparable treated emulsions. Although not generally strongly perceived in aromas of fish oils, the concentration of (*E,Z,Z*)- and (*E,E,Z*)-2,4,7-decatrienals varied widely from 3−4 ppb for the raffinose emulsion to 186−636 ppb for the sucrose emulsion.

Influence of Fructose on the Rate of Retroaldol Conversion of (*E,Z*)-2,6-Nonadienal in Aqueous Solutions. Because aroma assessments had indicated that some polyol treatments exhibited less extensive oxidative aroma deterioration (sucrose, raffinose, and mannitol) to yield green−fishy aromas rather than oxidized−painty aromas (control, fructose, and sorbitol), the observation suggested that the powerfully green (*E,Z*)-2,6-nonadienal (*2*) was not being extensively converted by retroaldol degradation (*44*) in the presence of polyols.

Results of a trial employing fructose (16%; similar to model emulsions) in a solution containing (*E,Z*)-2,6-nonadienal (600 ppm) at pH 6.5 at 21 °C compared to a control sample without fructose are shown in **Figure 1**. These data showed that the presence of the sugar slightly suppressed the retroaldol conversion of (*E,Z*)-2,6-nonadienal to (*Z*)-4-heptenal during the initial 24 h period, which coincided with the length of the exposure employed for the emulsion oxidation study (**Table 2**). Furthermore, the data agreed with the limited suppression of the retroaldol formation of (*Z*)-4-heptenal by fructose in the model emulsion (**Table 2**; 28% reduction compared to the control). However, between 24 and 48 h of reaction time, the concentration of (*Z*)-4-heptenal rose notably in the control sample while

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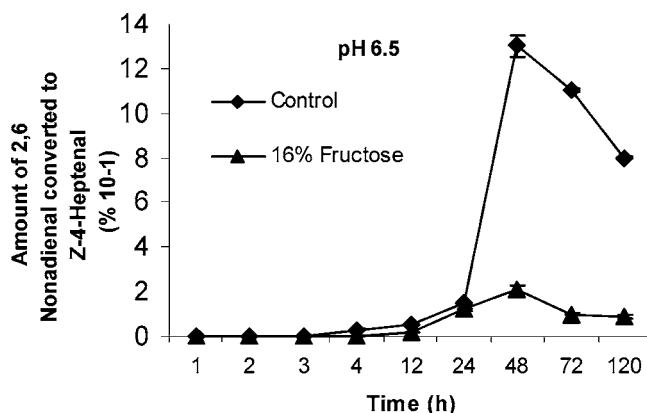
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Figure 1. Effect of fructose in aqueous solution (16%) on the retroaldol conversion of (*E,Z*)-2,4-nonadienal to (*Z*)-4-heptenal at pH 6.5 and 21 °C during holding for up to 120 h.
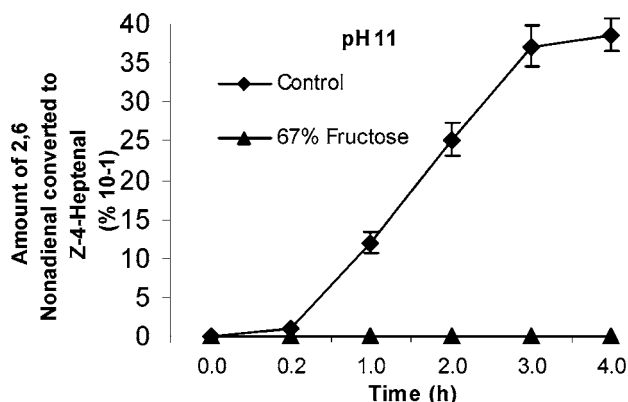

Figure 2. Effect of fructose in aqueous solution (67%) on the retroaldol conversion of (*E,Z*)-2,4-nonadienal to (*Z*)-4-heptenal at pH 11 and 21 °C during holding for up to 4 h.

Table 3. Influence of Fructose in Aqueous Solution on the Water Activity (a_w) of Model Systems Employed in Various Trials

sample	water activity[a] (a_w)
emulsions	
control (no added fructose)	0.980
+ fructose (16% in aqueous phase)	0.963
aqueous solutions	
+ fructose (16%)	0.966
+ fructose (67%)	0.777

[a] Standard deviation < ±0.001 for each sample.

that in the experimental fructose solution (16%) increased only slightly. These data indicated that competition between the retroaldol reaction and the hydration of the fructose ultimately resulted in a suppression of the formation of (*Z*)-4-heptenal, which amplifies fishy−burnt flavors in fish oils (*2*).

In another trial with a concentrated fructose aqueous solution (67%), the pH was adjusted to 11 to provide more favorable retroaldol reaction conditions, and the conversion of (*E,Z*)-2,6-nonadienal (600 ppm) was completely inhibited in the fructose-containing solution during a 4 h reaction period, whereas the nontreated control sample exhibited rapid conversion of (*E,Z*)-2,6-nonadienal (**Figure 2**). Measurement of the water activities of some of the model systems employed in the various trials showed that a fructose concentration to 67% greatly suppressed the water activity (**Table 3**) and provided conditions which inhibited the retroaldol conversion of (*E,Z*)-2,6-nondienal to (*Z*)-4-heptenal (**Figure 2**).

The data in **Table 3** also showed that 16% fructose solute in model systems slightly suppressed the water activity, and hence the molecular mobility likewise would have been expected to be only slightly suppressed. Therefore, the water activity data supported an interpretation that restricted molecular mobility provided by elevated concentrations of sugars and polyhydric alcohols (*48*) retards the water-requiring retroaldol decomposition of 2-alkenals. Even though apparently only a slight suppression of the retroaldol reaction occurred for the conditions employed in the fish oil emulsion studies, food emulsion formulations or surimi seafood analogues (*49*) containing greater concentrations of polyol solutes in the aqueous phase (>16%) likely would possess reaction-inhibiting conditions that would influence the relative contributions of 2-alkenals and their retroaldol products in lipid oxidation flavors.

Influence of Inclusion of Selected Sugars and Polyols in Combination with Phenolic Antioxidants on the Oxidation of Fish Oil Emulsions Held under Light (2550 lx) for 24 h at 5 °C. Results of studies introducing a combination of selected polyols and phenolic antioxidants on the oxidation quality of fish oil emulsions are shown in **Table 4**. When phenolic antioxidants were introduced alone, substantial reductions (51−79%) in peroxide values compared to controls were observed, and oxidation-suppressed, green to green−fishy aromas developed rather than the oxidized−painty aromas observed for untreated controls. Generally, TBHQ was the most effective antioxidant, BHT the next most effective, and α-tocopherol the least effective. Quantitative data for volatile aroma compounds from the TBHQ series that exhibited the most efficient antioxidant effects are shown in **Table 5** to illustrate the suppression of volatiles observed for the emulsions. Measurement of volatile oxidation compounds for the other treated emulsions also generally showed distinct reductions in concentrations of volatiles compared to the control (data not shown), especially those associated with painty−oxidized aromas [hexanal, (*Z*)-4-heptenal, 2,4-heptadienals].

Introduction of polyols along with phenolic antioxidants unpredictably influenced peroxide values for fish oil emulsions, and some additions (α-tocopherol and TBHQ) resulted in slightly greater reductions than were observed for the corresponding samples containing only the phenolic antioxidant (**Table 4**). However, addition of polyols along with BHT consistently interfered with the antioxidant activity of BHT and resulted in slightly reduced antioxidant activities (lower peroxide values) than were obtained when BHT was added alone.

Thus, instead of an anticipated synergistic antioxidant activity between polyols and phenolic antioxidants, total activity for polyol and phenolic antioxidant combinations in emulsions was frequently lower than seen when the phenolic antioxidant was used singly in emulsions (**Table 4**). This suggested that molecular-level interactions between polyol and phenolic compounds occurred in the aqueous phase of the fish oil emulsions, and these interactions often caused net losses of antioxidant activity compared to those expected for phenolic compounds alone or that expected by an additive synergism between polyols and phenolic antioxidant.

Several groups (*50, 51*) have shown that the antioxidant activity of phenolic antioxidants is greatly reduced in the presence of protic solvents. This alteration of antioxidant activity has been attributed to hindrance of the H atom donating activity of the phenolic antioxidant compounds by the hydrogen bond accepting activity of the protic organic compounds, and therefore the antioxidant efficiencies of phenolic compounds become diminished in their presence. Because of the highly hydroxylated

Table 4. Influence of Inclusion of Selected Sugars and Polyhydric Alcohols (4.23% of Formula; 16% in Aqueous Phase) upon the Oxidation of Fish Oil Emulsions Containing Phenolic-type Antioxidants (α-Tocopherol, 1000 ppm; BHT, 200 ppm; or TBHQ, 200 ppm; in Lipid Phase) after Holding under Light (2550 lx) for 24 h at 5 °C

	peroxide value		
emulsion treatment	μM hydroperoxide/ mg of fish oil[a]	change relative to control (%)	headspace aroma description
control (no treatment)	6.8	— [b]	oxidized–painty
α-tocopherol only (1000 ppm)	3.3	51 (−)[c]	slightly green
+ fructose	2.6	61 (−)	slightly green
+ sorbitol	2.6	62 (−)	green–fishy
+ Sucrose	3.2	53 (−)	green–fishy
+ raffinose	3.1	54 (−)	slightly green
+ mannitol	3.5	48 (−)	slightly green
control (no treatment)	4.0	—	oxidized–painty
BHT only (200 ppm)	0.97	76 (−)	green–fishy
+ fructose	1.2	70 (−)	painty
+ sorbitol	1.2	70 (−)	painty
+ sucrose	1.05	74 (−)	green–fishy
+ raffinose	1.08	73 (−)	green–fishy
+ mannitol	1.3	68 (−)	green–fishy
control (no treatment)	6.8	—	oxidized–painty
TBHQ only (200 ppm)	1.4	79 (−)	slightly green
+ fructose	0.74	89 (−)	slightly green
+ sorbitol	1.8	74 (−)	green
+ sucrose	1.85	73 (−)	green–fishy
+ raffinose	0.76	89 (−)	slightly oxidized–fishy
+ mannitol	1.65	76 (−)	green–fishy

[a] Average of four replicate analyses; standard deviation < ±0.3 for all determinations. [b] Not applicable; antioxidants or polyols were not added to control samples. [c] Extent (percent) and direction of change compared to an untreated control; (+) = increase; (−) = decrease.

Table 5. Effect of Inclusion of Selected Sugars and Polyhydric Alcohols (4.23% of Formula; 16% in Aqueous Phase) upon Formation of Volatile Oxidative Compounds When Combined with TBHQ (200 ppm) in Fish Oil Emulsions and Held under Light (2550 lx) for 24 h at 5 °C

	fish oil emulsion treatment											
	TBHQ		TBHQ + fructose		TBHQ + sorbitol		TBHQ + sucrose		TBHQ + raffinose		TBHQ + mannitol	
volatile compound	ppb[a]	% change[b]	ppb	% change	ppb	% change	ppb	% change	ppb	% change	ppb	% change
hexanal	460[c]	50 (−)	162	82 (−)	146	84 (−)	284	69 (−)	199	78 (−)	233	74 (−)
(E)-2-hexenal	233	26 (−)	113	63 (−)	126	60 (−)	53	83 (−)	186	40 (−)	78	75 (−)
(Z)-4- heptenal	160	49 (−)	103	67 (−)	146	54 (−)	144	54 (−)	185	41 (−)	128	59 (−)
(Z)-3-hexen-1-ol	42	67 (+)	20	30 (+)	70	80 (+)	22	36 (+)	83	83 (+)	25	44 (+)
2-octenal	395	28 (−)	513	6 (−)	526	3 (+)	488	10 (−)	615	11 (+)	523	4 (−)
(E,Z)-2,4-heptadienal	18	44 (−)	14	56 (−)	16	50 (−)	22	31 (−)	59	46 (+)	12	63 (−)
1,5-octadien-3-ol	23	77 (−)	9	90 (−)	15	85 (−)	19	81 (−)	47	53 (−)	13	86 (−)
(E,E)-2,4-heptadienal	14	60 (−)	9	74 (−)	12	66 (−)	13	63 (−)	46	24 (+)	12	66 (−)
(E,Z)-3,5-octadien-2-one	14	74 (−)	12	77 (−)	19	64 (−)	14	74 (−)	98	46 (+)	16	70 (−)
(E,E)-3,5-octadien-2-one	6	60 (−)	13	13 (−)	2	87 (−)	8	47 (−)	67	78 (+)	2	87 (−)
(E,Z)-2,6-nonadienal	4	90 (−)	10	76 (−)	6	85 (−)	4	91 (−)	39	7 (+)	8	81 (−)
(E,Z)-2,4-decadienal	30	50 (+)	27	44 (+)	8	47 (−)	68	78 (+)	28	46 (+)	137	89 (+)
(E,E)-2,4-decadienal	15	69 (−)	117	58 (+)	20	59 (−)	88	44 (+)	120	59 (+)	145	66 (+)
(E,Z,Z)-2,4,7-decatrienal	22	15 (−0	219	88 (+)	11	58 (−)	48	46 (+)	87	70 (+)	164	84 (+)
(E,E,Z)-2,4,7-decatrienal	22	88 (−)	168	12 (−)	18	91 (−)	23	88 (−)	88	53 (−)	83	56 (−)

[a] Concentration of volatile compound in treated emulsion. [b] Extent (percent) and direction of change relative to control, nontreated sample; (−) = decrease; (+) = increase. [c] Concentrations of volatiles are within ±20% method variability.

molecular features of the sugars and polyhydric alcohols employed in this study, it is proposed that such H-bonding molecular interactions are responsible for the general lack of antioxidant synergism observed for combination of phenolic antioxidants and polyols in fish oil emulsions.

The higher degree of antioxidant interference by polyols for α-tocopherol and BHT compared to TBHQ (**Table 4**) theoretically could be related to the polarity of the respective phenolic antioxidants. For the more polar TBHQ, greater concentrations would be expected to reside near the oil−water interface because of low oil solubility than would be expected for the less polar, more oil-soluble α-tocopherol and BHT, which would be more

extensively dispersed in the oil phase (47, 52, 53). As a consequence, the net interference with phenolic antioxidant activity by polyols would be expected to be less for TBHQ because of its higher concentration of the oil−water interface than that found for either α-tocopherol or BHT.

Summary and Conclusions. Model fish oil emulsions containing polyols at 16% of aqueous phase distinctly reduced peroxide values (−11 to −18%) compared to control samples. Viscosities of model emulsions containing polyols varied widely; mannitol exhibited the highest emulsion viscosity but had the lowest antioxidancy, whereas fructose had the lowest viscosity but had the highest antioxidant activity. Higher water solubilities

of polyols were associated with more effective antioxidant activities (peroxide values), and therefore the data supported a hypothesis that either or both free radical-scavenging or transition state metal chelation activities were provided by polyols in fish oil emulsions.

Peroxide values were not always good indicators of the extent of oxidative deterioration in fish oil emulsions as indexed by aroma quality because the degree of suppression of peroxide values did not readily correlate with the extent of oxidation indicated by the aroma (green to fishy−green to painty−oxidized). Polyols retarded the water-requiring retroaldol decomposition of (*E,Z*)-2,6-nonadienal to (*Z*)-4-heptenal in the model systems, and the reaction may be involved in some suppression of fishy flavors in emulsions.

Combinations of phenolic antioxidants (α-tocopherol, TBHQ, and BHT) and polyols in fish oil emulsions revealed that polyols antagonized the antioxidant activity of phenolic antioxidants. Molecular-level interactions involving H-bonding activity between polyols and phenolic antioxidants in the aqueous phase were proposed to interfere with H-donating activities of the phenolic antioxidants, which in some cases reduced the antioxidancy of combinations compared to that of the phenolic compounds alone.

These results indicated that some polyols (fructose, sorbitol; for chelating transition state metals, scavenging •OH, and reducing the diffusion of oxygen) in combination with an oil soluble antioxidant, for example, α-tocopherol, might be applicable for delaying the development of objectionable flavors in fish oil emulsions contained in foods, such as beverages, salad dressing, and mayonnaise. However, it remains to be determined whether polyol−phenolic antioxidant systems will perform in emulsions as well as the polyol−lecithin antioxidant systems described by Schroder and Muffett (*14*).

Light was used in this study as the initiator (abstraction of hydrogen from lipid molecule or photosensitized oxidation type II through singlet oxygen generation) of lipid oxidation to accelerate the oxidation of fish oil. It is also theorized that without light as the initiator of lipid oxidation, polyols in fish oil emulsions will also prolong fish oil stability under darkened conditions. However, the effect of darkened conditions on fish oil emulsion stability needs to be further investigated and compared with those observed for lighted conditions. Because incorporation of high concentrations of polyols in emulsions increases the viscosity and suppresses the retroaldol reaction, polysaccharides could be examined and included in model emulsion systems.

ACKNOWLEDGMENT

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