Chemical and Sensory Characteristics of Stored Menhaden Oil/ Soybean Oil Blends

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The purpose of this study was to determine the feasibility of increasing the consumption of dietary ω-3 fatty acids by incorporating menhaden oil into a French-type salad dressing. Menhaden/soybean oil blends of 10, 20 and 30% menhaden oil (w/w) were used to prepare an emulsified French salad dressing. The oil blends and salad dressings were stored at 22°C in the dark for 20 wk. The fatty acid profile, peroxide value, and anisidine value were determined. The salad dressings also were evaluated by a sensory panel for flavor, aroma, and aftertaste. The ω-3 fatty acids were stable over time under these storage conditions. Peroxide values rose slowly and consistently over time reaching higher values when more menhaden oil was added. Peroxide values were also higher in the oil blends which were stored with air in the headspace and not flushed with argon. Anisidine values also were higher with each addition of menhaden oil but did not change over time except for the 100% menhaden oil which was stored in air. After eight weeks the sensory panel rated the salad dressing which contained menhaden oil as lower than the ones which did not contain menhaden oil. While a significant amount of ω-3 fatty acids may be incorporated into foods by the addition of menhaden oil, the development over time of off-flavors must be controlled.

KEY WORDS: Menhaden oil, oxidation, sensory characteristics.

With GRAS (Generally Recognized As Safe) status recently granted to hydrogenated and partially hydrogenated menhaden oil by the U.S. Food and Drug Administration and the petition for approval of menhaden oil pending, renewed interest exists for studying the incorporation of this oil into foods. Menhaden are small, oily, herring-like fish that live at or near the surface of large bays and along the Atlantic and Gulf coasts. These fish represent approximately 98% of the U.S. marine oil production. Menhaden oil, which should be able to compete economically with vegetable oils, has the added advantage of being a rich source of the nutritionally important ω-3 fatty acids (1). Typical menhaden oil contains 13% eicosapentaenoic acid (EPA) and 8% docosahexaenoic acid (DHA) (2), the two ω-3 fatty acids which are thought to be primarily responsible for the favorable alterations in blood parameters (3).

Fish oil can either be incorporated into food systems after partial hydrogenation or with the fatty acid composition unaltered. In its hydrogenated form, it currently is used in salad oils, frying fats, table spreads, table margarines, and industrial margarines in Europe (4). However, hydrogenation destroys some of the ω-3 fatty acids. On the other hand, fish oil that has not been hydrogenated develops fishy off-flavors which are a major problem in the utilization of fish oil in food products. Although improved deodorization processes have been developed, fishy off-flavors continue to limit the use of menhaden oil. Trimethylamine, as well as compounds from oxidizing polyunsaturated fatty acids, have been thought to be responsible for these off-flavors (5). However, it may be possible to replace some of the vegetable oil in certain food systems with marine oil without affecting the sensory characteristics of the food. Vegetable oils contain natural antioxidants which may give some additional protection to fish oils (6).

The purpose of this study was to determine the effect of substituting menhaden oil for soybean oil in stored soybean oil/menhaden oil blends and in French salad dressing made with these blends.

EXPERIMENTAL PROCEDURES

Sample preparation and storage. Fresh specially processed menhaden oil (SPMO) that was refined, bleached, deodorized, and treated with 0.1% tenox (Tenox 20 a, Eastman Kodak, Kingsport, TN) was obtained from Zapata Haynie Corporation (Reedville, VA). SPMO contains approximately 17% EPA and 10% DHA. Soybean oil (Crisco, Procter and Gamble, Cincinnati, OH) was purchased locally. Three blends of 10%, 20% and 30% menhaden oil (w/w) were made. Approximately 25 mL of each blend and controls of 100% soybean oil and 100% fish oil were stored in 30-mL, airtight glass vials. The samples were flushed with argon before sealing the vials. A French salad dressing, which contained approximately 65% oil, was made from each oil and each blend. The formula for the French salad dressing was: 334 g vegetable oil, 66 g lemon juice, 100 g apple cider vinegar, 1.06 g paprika, 1.12 g dry mustard, 2.84 g iodized salt, 25.08 g granulated sugar, 1.70 g xanthin gum, and a few grains of cayenne pepper. The salad dressing samples were stored in 15-mL glass vials flushed with argon. Samples of salad dressing for sensory analysis were stored in half-pint jars with argon in the headspace. All samples were stored in an incubator at 22°C in the dark for 20 wk. A new vial or jar was opened each time the objective and sensory tests were run. In order to study the effect of oxygen on stored samples, the blends and controls were stored in bottles not flushed with argon. The same bottle was opened each time the tests were run. All tests were done in duplicate.

Gas chromatographic analysis. The fatty acids in the oil samples were converted to methyl esters using a procedure adapted from Luddy *et al.* (7). A 0.85-mL lipid sample was mixed with 5 mL anhydrous methanol and 2 mL 0.8 N sodium methoxide. The solution was vortexed and heated at 68°C for 15 min. Petroleum ether was used to separate the lipid fraction. The same methylation procedure was used for the salad dressings, but the lipid was extracted first using a chloroform: methanol solution

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(2:1 v/v). The methyl esters were analyzed using a Shimadzu gas chromatograph, model GC-9A, with a SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, 30 m × 0.32 mm inner diameter, 0.25 micrometer film). Temperature programming was used to increase resolution. The initial temperature of the column was 150°C. The temperature increased at the rate of 2°C/min until a final temperature of 200°C was attained. Total elution time was 35 min. The flow rate through the column was 20 cm/sec. Split injection (20:1) was used with a sample size of 0.5 microliters. The flow rate of helium, the carrier gas, was 1 mL/min. The makeup gas consisted of helium, hydrogen (flow rate =30 mL/min), and air (flow rate =300 mL/min). The flow rate of the makeup gas through the flame ionization detector (FID, 4 × 10E -11 Attenuation Full Scale) was 50 mL/min.

A vegetable standard, RM1, and a marine standard, PUFA1 (Supelco), were run before each series of methyl esters was analyzed.

Peroxide value. The peroxide value (PV) was determined by the AOAC method 28.026 (8).

*Anisidine value.*The anisidine value was determined by using the method recommended by the International Association of Fish Meal Manufacturers (9). Reagents used were of analytical grade. Conjugated dienals react with *p*-anisidine to form a yellow pigment which was measured using an ultraviolet/visible spectrophotometer, Bausch and Lomb Spectronic 2000.

Sensory analysis. A thirteen-member panel evaluated the salad dressing for odor, flavor, and aftertaste after 1,2,4,6 and 8 wk of storage. An unstructured 15-cm scale, anchored 1 cm from each end, was used to record the panelists' scores of the samples. The anchor words for both aroma and flavor were "fresh" and "rancid". The anchor words for aftertaste were "none" and "strong". The samples were presented simultaneously and coded with a random three-digit code. Warm water was available for rinsing the mouth, and crackers were available for cleaning the palate between samples. All sensory analyses were conducted in the sensory analysis laboratory of the Human Nutrition and Foods Department at Virginia Polytechnic Institute and State University, Blacksburg, VA.

A training session was held prior to the first day of sensory analysis to familiarize the panelists with the overall procedures to be used and to teach them how to judge the freshness of salad dressing. In addition, each time the panel met, fresh, slightly rancid, and very rancid oil samples were available for panelists to smell to acquaint them with typical rancid odors.

RESULTS AND DISCUSSION

Fatty acid composition. Fatty acid compositions of the salad dressings made with fish oil blends are given in Table 1. As the percentage of menhaden oil increased, the amount of ω-3 fatty acids also increased. Slight variations in fatty acid content were seen over time but no significant changes were evident. After 20 wk of storage, the total EPA and DHA content of the 100% menhaden oil and the salad dressing made with 100% menhaden oil decreased by 0.27% and 0.38%, respectively. Under these storage conditions and for this period of time, these highly unsaturated fatty acids were stable.

Oxidative tests. Peroxide and anisidine values were determined and used to measure the level of oxidation in the oil blends. The peroxide values of the oil blends, which had been flushed with an inert gas, rose slowly and consistently throughout the 20 wk of the study (Table 2). Only at wk 20 did the peroxide values for the 20% and 30% blends begin to increase at a faster rate than the 10% blend or the control with no menhaden oil. The 100% menhaden oil also began to increase more rapidly after 20 wk of storage.

The effect of oxygen on the blends was evident by comparison of the peroxide values of the oil blends which were not flushed with an inert gas to those which were flushed. After 15 wk of storage, the peroxide values began to increase and at 20 wk had almost doubled from the values observed at 15 wk (Table 3).

Freshly refined oils should have a peroxide value of less than 1 meq/kg. Use of peroxide value to determine the quality of an oil is not clear. Flavor deterioration in soybean oil may occur at relatively low PV (5-10 meq/kg)

TABLE 2

Peroxide Values of Fish Oil/Soybean Oil Blends Flushed with Argon and Stored in the Dark at 22°C[a]

Fish oil (%)	0[b]	1	4	6	8	10	15	20
0	0.4[c]	1.0	0.3	0.9	1.8	2.1	2.0	3.3
10	0.7	1.6	1.3	2.2	3.3	2.6	2.7	3.0
20	0.4	1.7	1.4	2.2	2.2	2.6	2.7	5.2
30	0.4	1.8	1.5	3.3	4.0	2.9	4.1	6.0
100	0.2	1.2	2.1	3.5	4.6	4.0	4.8	7.0

[a]Average from duplicate runs.

[b]Time span is 0-20 wk.

[c]Peroxide values (meq/kg).

TABLE 1

Fatty Acid Composition of Salad Dressings Made with Fish Oil/Soybean Oil Blends[a]

	0[b]	10	20	30	100
C 18:1	21.4 ± 0.63	21.1 ± 0.30	20.0 ± 0.58	19.4 ± 0.24	10.6 ± 0.69
C 18:2	57.8 ± 0.82	2.8 ± 0.66	48.8 ± 1.35	43.4 ± 0.45	1.4 ± 0.21
C 18:3	7.4 ± 0.06	7.1 ± 0.11	6.3 ± 0.01	5.6 ± 0.04	0.81 ± 0.04
C 20:5	0.0	1.2 ± 0.01	2.5 ± 0.30	4.1 ± 0.13	14.8 ± 0.06
C 22:6	0.0	0.6 ± 0.03	1.1 ± 0.13	1.6 ± 0.12	7.4 ± 0.81

[a]Mean % of total fatty acid content ± SD, n = 2.

[b]Fish oil (%) in 0-100.

TABLE 3

Peroxide Values of Fish Oil/Soybean Oil Blends Capped and Stored in the Dark at 22°C[a]

Fish oil (%)	0[b]	1	4	6	8	10	15	20
0	0.4[c]	0.9	1.0	1.3	1.6	1.8	2.6	5.0
10	0.7	1.7	2.3	2.6	3.1	4.1	5.5	8.5
20	0.4	1.3	2.5	2.7	3.4	3.7	5.3	9.5
30	0.4	1.0	1.4	2.2	2.2	3.3	6.4	13.0
100	0.2	1.0	9.5	18.6	27.7	36.0	45.1	62.0

[a]Average from duplicate runs.

[b]Time span is 0-20 wk.

[c]Peroxide values (meq/kg).

TABLE 5

Anisidine Values of Fish Oil/Soybean Oil Blends Capped and Stored in the Dark at 22°C[a]

Fish oil (%)	0[b]	2	4	6	8	10	15	20
0	1.3[c]	1.0	1.3	1.7	1.6	1.5	1.6	1.8
10	4.3	3.5	4.6	4.2	3.9	4.2	4.1	4.4
20	5.5	4.3	5.1	4.9	4.4	4.7	5.1	5.1
30	6.7	5.2	5.7	5.9	5.3	5.7	6.0	6.4
100	16.3	14.4	16.2	21.6	28.7	29.0	38.8	50.1

[a]Average from duplicate runs.

[b]Time span is 0-20 wk.

[c]Anisidine values.

which corresponds to a level of oxidation of 0.1% or less (10). Robards and co-workers (11) state that a peroxide value greater than 2.5 meq/kg may indicate oxidation and a lack of stability in fried chips. In addition, a peroxide value greater than 7.5 meq/kg may be indicative of sufficient breakdown of peroxides to aldehydes and could produce the rancid flavor in chips. Snyder *et al.* (12) reported that several volatile flavor components — pentane, hexanal, 2-heptenal, 2,4-heptadienal, and 2,4-decadienal — were present in soybean oil with peroxide values between 2 and 10 meq/kg. Warner *et al.* (13) found peroxide values to be of limited usefulness in predicting oxidative deterioration of crude and refined soybean oil.

The anisidine value is a relative measure of the secondary products of oxidation. It specifically measures conjugated dienals, particularly 2-alkenals. In this study the samples flushed with argon showed no differences in anisidine value from the beginning of the study until 20 wk (Table 4). Anisidine values rose with each additional amount of fish oil but then did not change. The same results were obtained with the samples stored in air, except for the 100% menhaden oil sample (Table 5). At wk 6 the anisidine values began to increase and continued to increase during the remaining period of the study.

Like peroxide value, the usefulness of the anisidine value in predicting the quality of oils remains a matter of debate. Typically the anisidine value will increase as aldehydes are produced and then decrease when the aldehydes reach a certain level and, subsequently, are further oxidized or participate in dimerization or condensation reactions (14). Robards *et al.* (11) state that the anisidine value does not correlate well with the sensory analysis of oils. The anisidine test has superseded the benzidine test, but neither test is intended to provide a quantitative content of carbonyl compounds. The flavor threshold of various carbonyl compounds differs greatly and makes correlation with sensory evaluations difficult. However, List and co-workers (15) found a significant correlation (-0.68) between anisidine values of salad oils from sound soybeans and their flavor scores. Anisidine values of sound oils were lower than for damaged oils at comparable stages of processing; however, little reduction of anisidine value occurred after deodorization of damaged oils. Likewise Warner *et al.* (13) found that anisidine values of crude oils showed a low correlation with flavor scores. However, anisidine values of unaged, deodorized oil had a significant correlation with flavor scores.

In this study the peroxide value seemed to be a more sensitive measure of oxidation than anisidine value. Anisidine values reflected the different levels of menhaden oil but did not change even though peroxide values rose. This was true for all the sample except for the 100% menhaden oil which was not protected from oxygen. In this sample both anisidine and peroxide values increased. Since anisidine value represents secondary oxidation products, it is possible that the oxidation level was still too low to be detected by this procedure. Only at higher peroxide values, such as those seen with the 100% menhaden oil, would changes in anisidine value reflect changes in oxidation.

Sensory analysis. Results of the sensory analysis are given in Tables 6,7, and 8. The lower the score, the fresher the salad dressing. After 6 wk of storage, the salad dressings made with 10% and 20% fish oil had similar ratings for aroma, aftertaste and flavor, with no significant differences among the samples. The 30% substituted

TABLE 4

Anisidine Values of Fish Oil/Soybean Oil Blends Flushed with Argon and Stored in the Dark at 22°C[a]

Fish oil (%)	0[b]	2	4	6	8	10	15	20
0	1.3[c]	1.0	1.5	1.6	1.4	1.5	1.5	1.3
10	4.3	3.1	3.8	4.0	3.9	4.1	4.1	3.4
20	5.5	4.7	5.2	5.4	4.9	5.6	4.8	5.1
30	6.7	6.8	5.6	5.5	5.4	6.1	5.5	5.6
100	16.3	14.0	16.6	17.2	16.7	17.7	15.8	16.4

[a]Average from duplicate runs.

[b]Time span is 0-20 wk.

[c]Anisidine values.

TABLE 6

Sensory Evaluation Scores[a] for Aroma of Salad Dressings Made with Fish Oil/Soybean Oil Blends (1-8 wk)

Fish oil (%)	1	2	4	6	8
0	4.0[a]	3.0[a]	2.8[a]	5.5[a]	4.3[a]
10	4.2[a]	3.5[a,b]	3.6[a,b]	6.6[a]	7.7[b]
20	4.7[a]	5.9[b]	5.9[a,b]	7.0[a]	7.9[b]
30	6.2[a]	4.1[a,b]	6.6[b]	—	—

[a]Mean of 13 scores. Means within a column with different letters are significantly different ($p < 0.05$). 1 = fresh, 14 = rancid.

TABLE 7

Sensory Evaluation Scores for Flavor of Salad Dressings Made with Fish Oil/Soybean Oil Blends (1-8 wk)

Fish oil (%)	1	2	4	6	8
0	$4.7^{a,b}$	3.8^{a}	4.1^{a}	5.4^{a}	4.5^{a}
10	$5.0^{a,b}$	3.9^{a}	4.9^{a}	6.1^{a}	7.9^{b}
20	3.8^{a}	5.5^{a}	$6.1^{a,b}$	6.3^{a}	7.8^{b}
30	6.9^{b}	5.4^{a}	8.3^{b}	—	—

[a]Mean of 13 scores. Means within a column with different letters are significantly different ($p < 0.05$). 1 = fresh, 14 = rancid.

TABLE 8

Sensory Evaluation Scores[a] for Aftertaste of Salad Dressings Made with Fish Oil/Soybean Oil Blends (1-8 wk)

Fish oil (%)	1	2	4	6	8
0	4.5^{a}	4.5^{a}	4.3^{a}	5.2^{a}	4.8^{a}
10	5.1^{a}	5.5^{a}	4.6^{a}	6.4^{a}	9.3^{b}
20	4.7^{a}	5.9^{a}	8.0^{b}	8.2^{a}	9.2^{b}
30	6.7^{a}	5.8^{a}	9.1^{b}	—	—

[a]Mean of 13 scores. Means within a column with different letters are significantly different ($p < 0.05$). 1 = fresh, 14 = rancid.

salad dressing was rated as being different from the control by wk 4 and was eliminated from the study. By the eighth week of storage, the salad dressings made with 10% and 20% menhaden oil were rated significantly higher than the soybean control for aroma, flavor, and aftertaste. The scores for aftertaste were higher than the scores for aroma and flavor. The addition of menhaden oil to the salad dressing seemed to impart a lingering unpleasant taste that the spices in the formula were not able to overcome. The sensory panel was able to detect differences in the salad dressings when the peroxide values for the stored oil blends were still low. The detection of rancidity by sensory panels is usually more sensitive than objective measurements.

Nutritional implications. The salad dressing made with 30% menhaden oil contained approximately 6% ω-3 fatty acids. The salad dressing is 65% oil, so a 100-g serving would provide about 4 g of ω-3 fatty acids. At the 10% level of replacement a 100-g serving would provide about 1.3 g of ω-3 fatty acids. This is about the same amount of ω-3 fatty acids as provided by a serving (100 g) of a fatty fish such as Albacore tuna or salmon and considerably more than that provided by lean fish such as flounder (0.2 g/serving) or halibut (0.4 g/serving). While 100 g (3.5 oz) of salad dressing is more than one serving, this salad dressing could be a significant source of ω-3 fatty acids.

Further research needs to be done on the development of off-flavors in foods which contain menhaden oil. The source of these off-flavors, which become evident at low peroxide values, needs to be determined. It may be feasible to develop a more effective antioxidant system or to mask the off-flavor by using marine oil in strongly flavored food systems.

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