NOAA TECHNICAL MEMORANDUM NMFS—SEFC 213

STORAGE STABILITY OF STEAM—DEODORIZED MENHADEN OIL IN SOFT GELATIN CAPSULES

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National Marine Fisheries Service
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U.S. DEPARTMENT OF COMMERCE
C. William Verity, Secretary
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
William E. Evans, Administrator
NATIONAL MARINE FISHERIES SERVICE
James W. Brennan, Assistant Administrator for Fisheries

INTRODUCTION

There is currently a great deal of interest in the value of omega-3 fatty acids in preventing coronary heart disease, autoimmune diseases and allergic reactions, arterial hypertension, and a number of other disease states. Much of the research done to date has been carried out with poorly defined and inconsistent test materials. In order to facilitate the evaluation of omega-3 fatty acids in health and disease, an interagency program called the Fish Oil Biomedical Test Materials Program (BTM) was established by a memorandum of understanding between the National Oceanic and Atmospheric Administration (NOAA)/National Marine Fisheries Service (NMFS) and the National Institutes of Health (NIH)/Alcohol, Drug Abuse, Mental Health Administration (ADAMHA).

The role of the NMFS in this program is to produce a long-term, consistent supply of chemically well defined, quality assured fish oil test materials. These test materials are supplied to researchers approved through the NIH Fish Oil Test Materials Advisory Committee for use in laboratory and clinical trials. All test materials are provided with quality assurance data which quantitatively define the lipid composition, the presence of any oxidation products, and non-lipid components, including environmental contaminants.

Because omega-3 fatty acids and their derivatives are highly unsaturated, they are extremely susceptible to oxidative deterioration; thus, the quality assurance data provided indicates the composition of the oil at the time it is shipped to the recipient researchers. It does not guarantee the quality of the test materials at the time they are used, since that depends on the length of time they are stored and under what conditions they are utilized. To aid in maintaining the quality of the test materials, the Charleston Laboratory is subjecting each type of test material produced to a formal stability study. From this information, recommendations can be made on how best to store the materials and for what length of time they can be expected to maintain the composition stated in the quality assurance report.

The materials currently being produced include both bulk and encapsulated steam- and vacuum-deodorized menhaden oil, omega-3 ethyl ester concentrates produced from vacuum-deodorized menhaden oil, and purified omega-3 esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

This report summarizes the results of a 12-month storage stability study on gelatin-encapsulated steam-deodorized menhaden oil.

METHODS AND MATERIALS

Steam-deodorized, partially refined menhaden oil was obtained (Zapata-Haynie Corp.)[1], packaged under nitrogen in food grade 55 gallon drums with antioxidants, alpha-tocopherol and tert-butyl hydroquinone (TBHQ), added to stabilize the product. Alpha-tocopherol (Eastman Chemical Co.) was added at a concentration of 1 g/kg. Tenox 20A (Eastman Chemical Co.) was added at a concentration of 1 g/kg to provide TBHQ at a level of 0.02%, the FDA allowable limit for food products.

The oil was encapsulated by a commercial encapsulator in soft gelatin capsules (No. 20 oblongs) containing 1 g oil/capsule. Titanium dioxide was added to the gelatin formulation to make the capsules opaque. The capsules were washed with hexane and dried according to industry standards and were packaged in brown glass bottles, 100 capsules/bottle, which were sealed with tamper-proof seals. The bottles were packaged 24 to a shelf-pack and three shelf-packs to a case.

Three vegetable oil placebos, grade A olive oil, food grade corn oil, and food grade safflower oil, were similarly encapsulated as No. 20 oblongs with titanium dioxide in the gelatin and packaged in brown glass bottles, 100 capsules/bottle, sealed with tamper-proof seals.

Homogeneity of the encapsulated menhaden oil was tested by analyzing 1 capsule from each of 10 bottles for fatty acid composition and peroxide value. The fatty acid composition varied by less than 1% between bottles. For peroxide value, which is the most sensitive assay to oxidative degradation, the relative standard deviation (RSD) was 11%, with the inherent variability of the assay being approximately 5% (Van Dolah and Galloway, 1988). Thus it was determined that sampling one bottle per time point would adequately represent the composition of the lot at that time point.

For the storage stability study, receipt of the test material and placebos at the laboratory was designated as time 0. At that time, 24 bottles of fish oil capsules were selected at random from 24 shelf packs (selected from 207 shelf packs by random number generation) for use in the study. Two bottles of each placebo were also randomly selected for use in the storage study. The capsules were stored at 5°C under ambient humidity conditions.

At time 0, one bottle was randomly selected and a complete quality assurance analysis was performed on the fish oil, including free fatty acids, fatty acid profile, iodine value, cholesterol, peroxide value, anisidine value, PCBs and pesticides, antioxidant content (tocopherol and TBHQ), moisture and sensory analysis (odor and flavor profiles). One bottle was subsequently analyzed every four weeks, through 52 weeks, for stability according to the analysis schedule in Table 1. Quarterly, additional analyses were carried out as shown in Table 1. All analyses were performed in triplicate. The remaining bottles were saved for long-term storage data.

[1] Mention of trade names does not imply an endorsement by NOAA/NMFS, but is provided for informational purposes only.

The vegetable oils were analyzed at time 0 and at 52 weeks storage for fatty acid composition, free fatty acids, and peroxide value only.

The methods of analysis used are those listed in the BTM Program quality assurance methods manual (1).

Table 1. Schedule of analyses performed to assess stability of encapsulated steam-deodorized menhaden oil.

Time (weeks)	0	4	8	12	16	20	24	28	32	36	40	44	48	52
EPA	x			x				x			x			x
DHA	x			x				x			x			x
TOTAL N-3	x			x				x			x			x
FREE FATTY ACIDS	x	x	x	x	x	x	x	x	x	x	x	x	x	x
IODINE VALUE	x													
CHOLESTEROL	x													
PEROXIDE VALUE	x	x	x	x	x	x	x	x	x	x	x	x	x	x
ANISIDINE VALUE	x			x				x			x			x
a-TOCOPHEROL	x			x				x			x			x
TBHQ	x			x				x			x			x
MOISTURE	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PCBs	x													
TOTAL DDT	x													
SENSORY ANALYSIS	x	x	x	x	x	x	x	x	x	x	x	x	x	x

RESULTS

Table 2 presents the composition of the encapsulated fish oil at time 0. There was little or no change in the composition or quality of the encapsulated oil over the 52 week duration of the study. The concentrations of EPA, DHA and total omega-3 fatty acids did not change over the 52 week storage of the material (Figure 1). Similarly, there was little or no change in the antioxidant content of the material (Figures 2-3), moisture (Figure 4), free fatty acids (Figure 5), peroxide value (Figure 6), or anisidine value (Figure 7). There was little, if any, increase in "total intensity" scores given by the sensory panel for odor and flavor during the study (Figure 8). Complete odor and flavor profiles at time 0 and at 52 weeks are compared in Figure 9.

The placebo oils demonstrated similar stability over the 52 week storage time (Table 3).

Table 2. Composition of soft gelatin encapsulated steam-deodorized menhaden oil at "time 0".

EPA, mg/g	118
DHA, mg/g	68
TOTAL n-3, mg/g	249
FREE FATTY ACIDS, %	nd[a]
CHOLESTEROL, mg/g	4.26
PEROXIDE VALUE	1.4
IODINE VALUE	178
ANISIDINE VALUE	18.2
ANTIOXIDANT CONTENT:	
a-TOCOPHEROL, mg/g	1.2
TBHQ, mg/g	0.12
MOISTURE, ug/g	200
PCB, ug/g	0.09
TOTAL DDT, ug/g	0.01
SENSORY ATTRIBUTES,	
0-15, 15 MAX INTENSITY:	
ODOR:	
TOTAL INTENSITY	3.64
BUTTERY	0.01
BEANY	0.11
RANCID	0.11
PAINTY	0.17
OXIDIZED	0.25
GRASSY	0.05
FISHY	0.96
BITTER	0.03
SWEET	0.84
FRUITY/MELON	0.21
BURNT	1.29
FLAVOR:	
TOTAL INTENSITY	3.76
BUTTERY	0.33
BEANY	0.27
RANCID	0.16
PAINTY	0.11
OXIDIZED	0.17
GRASSY	0.19
FISHY	2.08
BITTER	0.07
SWEET	0.73
FRUITY/MELON	0.14
BURNT	0.85
HELLIGE No.:	6

[a] not detected

Table 3. Composition of placebo oils at time 0 and 52-weeks storage.

	OLIVE		CORN		SAFFLOWER	
TIME, WEEKS	0	52	0	52	0	52
EPA, mg/g	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
DHA, mg/g	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL n-3, mg/g	7	7.6	9	9	1	1
FREE FATTY ACIDS, %	0.29	0.16	0.06	0.13	0.02	0.02
PEROXIDE VALUE	5.97	5.13	1.03	1.22	2.79	2.75
IODINE VALUE	90.8	nd	126.7	nd	139.9	nd

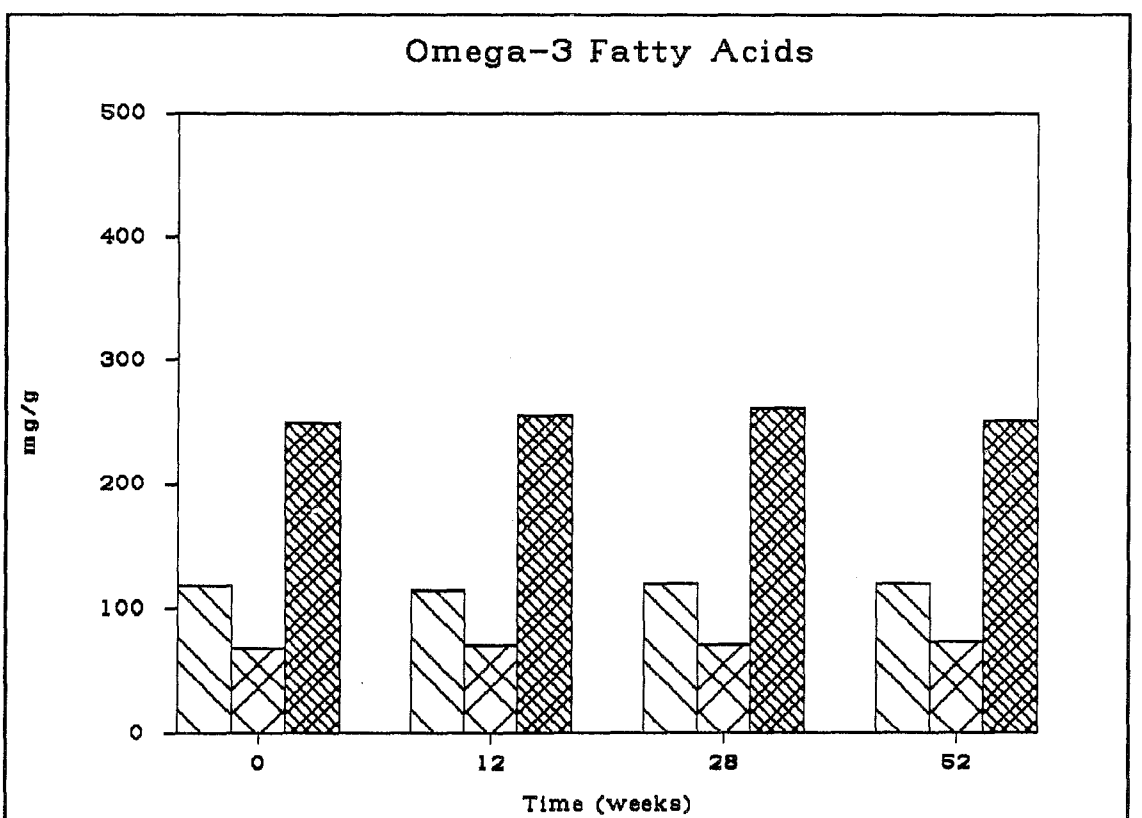

Figure 1. Concentrations of EPA, DHA, and total omega-3 fatty acids.

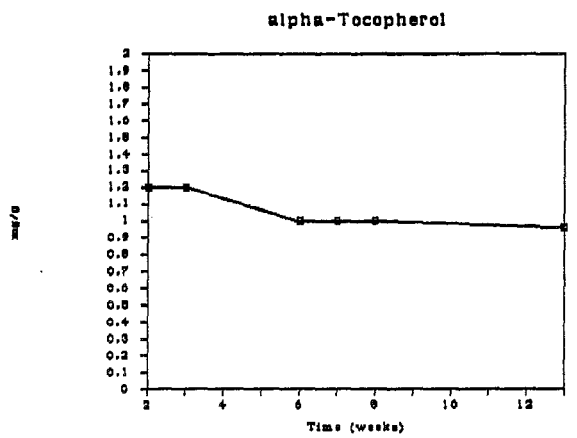

Figure 2. Alpha-tocopherol concentration.

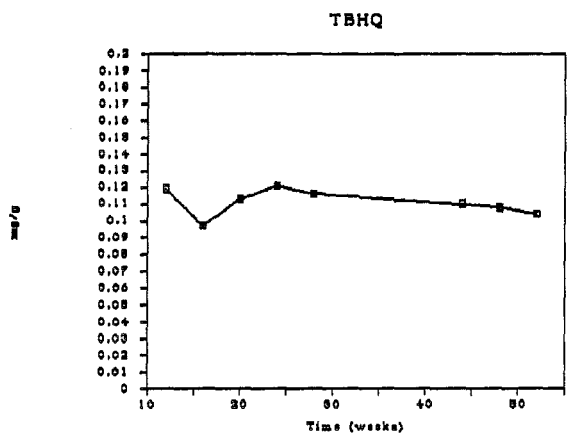

Figure 3. TBHQ concentration.

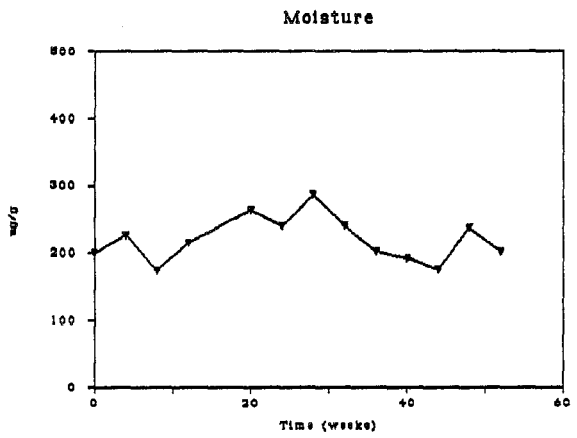

Figure 4. Moisture content.

Figure 5. Free fatty acid content.

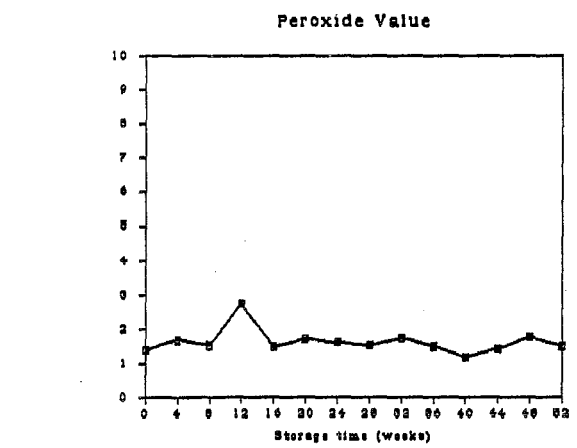

Figure 6. Peroxide Value.

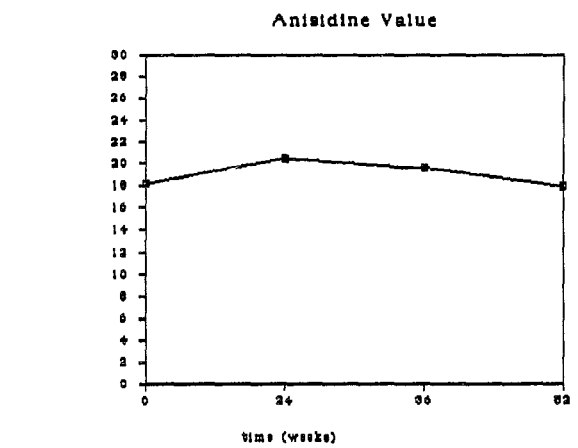

Figure 7. Anisidine value.

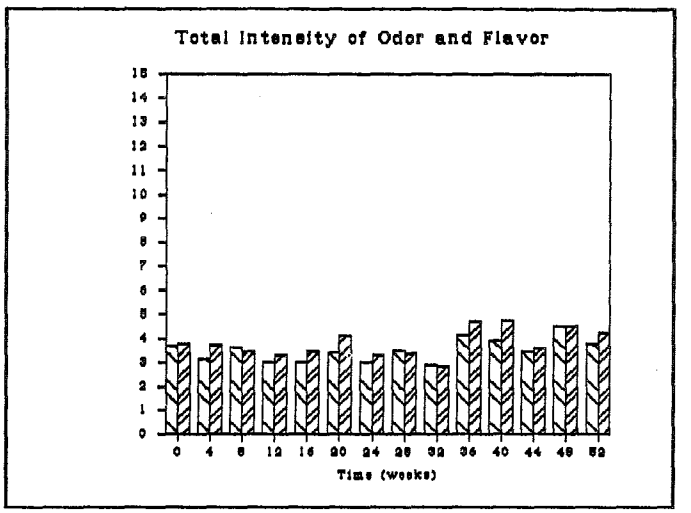

Figure 8. Total intensity scores for odor and flavor.

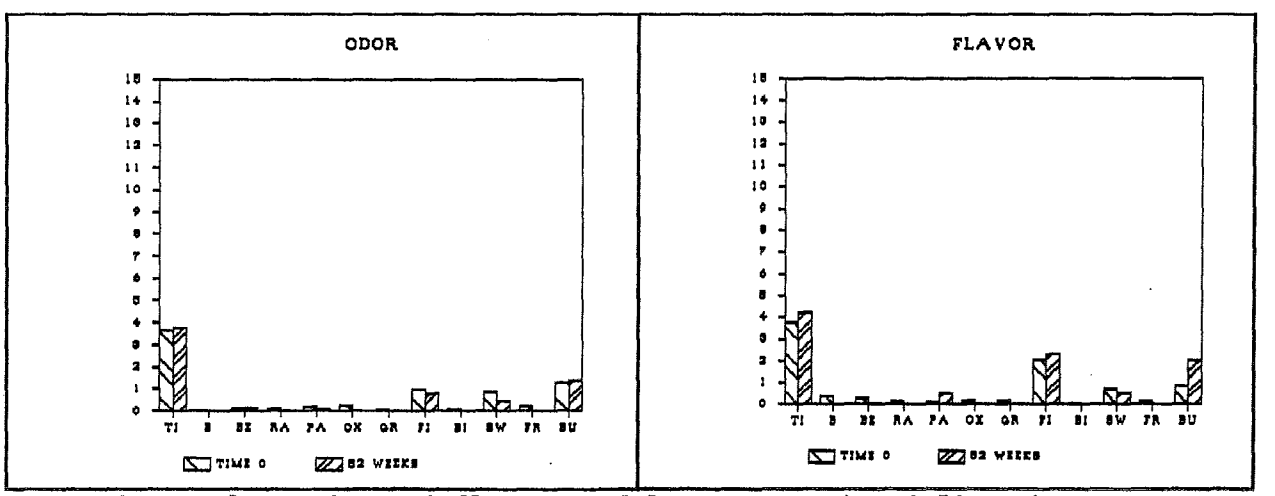

Figure 9. Complete odor and flavor profiles at time 0 and 52-weeks storage. TI total intensity; B buttery; BE beany; RA rancid; PA painty; OX oxidized; GR grassy; FI fishy; BI bitter; SW sweet; FR fruity; BU burnt.

DISCUSSION

Soft gelatin capsules are widely used in the pharmaceutical industry to provide a solid dosage form containing liquid medication. The predominant advantages of this mode of administration are accuracy and uniformity. The capsule is composed of gelatin, water and a plasticizer, usually glycerin or sorbitol. Water-immiscible liquids, water-miscible liquids containing <5% water, and dry powders can be encapsulated by this method.

The effects of temperature and humidity on the gelatin capsule therefore are well studied (2). For the unprotected capsule, low humidities (<20 R.H.), low temperatures (<2°C) and high temperatures (>37°C) or combinations of these will have transient effects, such as brittleness. As the humidity and temperature are moderated, the capsule will regain moisture in proportion to its gelatin and glycerin content.

High humidities (>60 R.H. at 21-23°C), however, can produce non-reversible effects on the unprotected capsule. As moisture is absorbed, the capsule becomes softer and tacky. As the humidity and temperature are moderated, the capsules may become cloudy and may stick together.

The capsules used in this study were held in the same conditions as those capsules stored for distribution by the BTM Program, 5°C and ambient humidity. The tamper-proof seal and glass bottle act as a moisture and oxygen barrier.

The assays used in the stability study of the encapsulated menhaden oil are assays routinely used by lipid chemists to determine oxidative degradation in an oil. The high degree of unsaturation in menhaden oil makes it extremely susceptible to oxidation, since oxidation reactions occur at the double bonds in the carbon chains of the fatty acids.

The iodine value is a measure of the total unsaturation of the oil. Peroxide value is a measure of the concentration of hydroperoxides present in the oil as a primary product of oxidation. The anisidine value measures aldehydes, which are a secondary oxidation product, derived from the intermediate hydroperoxides. All of these values remained constant throughout the 52 week study period.

Moisture was analyzed since the uptake of significant amounts of moisture from or through the gelatin capsule may lead to the production of free fatty acids by hydrolysis of the ester bonds in the triglyceride. Thus, free fatty acid content was also determined. Slight fluctuation in the moisture content of the oil may reflect variability between bottles. The slight decrease in the free fatty acid content reflects a modification to the method of analysis after the 16-week time point.

The concentrations of *a*-tocopherol and TBHQ were analyzed to determine if the antioxidant activity in the test material remained constant. The occurrence of oxidation in the oil may decrease the *a*-tocopherol content. The slight decrease in the *a*-tocopherol content over the 52-week period is within the precision limits of the assay.

An important component of the quality assurance carried out at the Charleston Laboratory is the analysis of the oil by a trained sensory panel. The sensory panel is frequently more sensitive to minor changes in oil quality than the chemical or instrumental methods. To produce a flavor and odor profile of the oil, the sensory panel ranks the oil on an unstructured scale 15-cm in length, where 0 represents "absence" of and 15 represents "very strong" presence of a flavor or odor characteristic, as defined by standard oils. Figure 8 demonstrates the mild responses of the panel to the steam-deodorized oil. There was very little, if any increase in the "total intensity" responses over the 52-week storage period. The only single odor/flavor characteristic which increased over the course of the study was a "burnt sugar" characteristic. The chemical identity of this component is not known. Analysis of volatile components in the test materials is presently in the development stage at the Charleston Laboratory.

Quarterly sampling of the stored steam-deodorized menhaden oil capsules is currently being carried out and analysis will continue at least through two years.

ACKNOWLEDGEMENTS

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