# Effects of Commercial Processing on the Fat-Soluble Vitamin Content of Menhaden Fish Oil

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**The effect of processing on the fat-soluble vitamin content of menhaden fish oil was studied. Menhaden oil samples were taken during various steps in commercial processing. Samples included a crude oil, a bleached oil, a refined oil, a refined then bleached oil, and a refined-bleached-deodorized-stabilized oil. The most processed oil had only about one-fifth as much vitamin A and vitamin $D_3$ as the crude fish oil. Vitamin E levels were decreased by about half as a result of processing. Bleaching the oil with Fuller's earth caused the major loss of retinols. Treating the fish oil with steam for several hours caused the major loss of vitamin $D_3$.**

**KEY WORDS: Commercial processing, fat-soluble vitamins, menhaden fish oil.**

Recent evidence indicates that marine fish oil in human diets is beneficial for lowering the incidence of cardiovascular disease (1). The effect is due to the n-3 polyunsaturated fatty acids in the oil (2), which also raise the possibility of increased autoxidation in the body (3). Marine fish oils provide naturally occurring antioxidants and other fat-soluble vitamins (4), but it is unknown how processing affects these nutrients.

Commercial processing of menhaden fish oil, as with most other oils, involves many steps (5). After the fish are caught and transported to the factory, they are cooked with steam to denature the protein and release bound water and fat (5,6). After the fish and liquid are separated, the fish are pressed to remove most of the liquid (oil and water). The pressed fish is called "press cake"; the liquid is called "press liquor".

The press liquor contains particles of fish; therefore, the liquid is centrifuged and the removed particles returned to the press cake (5,6). The press liquor is heated and centrifuged to separate the oil from the stickwater (water and smaller suspended fish particles). Stickwater is evaporated to increase the percent solids from near six percent to approximately fifty percent (6), after which it can be sold as fish solubles. However, most of the fish solubles are added back to the press cake to form "wholemeal".

The oil is then polished by adding water and centrifuging (5,6). At this stage, the oil is crude commercial fish oil. However, before the fish oil can be consumed, it must undergo further refining and processing to produce a more pure and stable product. The steps used in processing the oil include gum conditioning, neutralization, washing, drying, bleaching, filtration, and deodorization and stabilization.

Gum conditioning and neutralization are often done together and involve the addition of a sodium hydroxide solution to the heated oil. This neutralizes the free fatty acids and removes many of the phospholipids, trace metals, pigments, and oil-insolubles (7,8). The soap is removed by washing the oil with water and allowing the aqueous phase to settle and drain off.

The next step is bleaching which involves the use of bleaching clay (Fuller's earth) to remove pigments, oxidation products, trace elements, and any remaining soap (7,8). Partial hydrogenation is done at this point and involves the use of a nickel catalyst and the addition of hydrogen gas. At the present time, fish oil must be partially hydrogenated before it can be used in food products (7,8). Deodorization is the final step and is a distillation under vacuum, using steam (8).

The purpose of this work was to analyze for vitamins A, $D_3$ and E in samples of menhaden fish oil taken during various stages of commercial processing. This information showed how processing affected the fat-soluble vitamin levels.

## MATERIALS AND METHODS

*Fish oil samples.* Menhaden fish oil samples were supplied by the Zapata Haynie Corporation (Reedville, VA). Cooked menhaden fish were cold pressed to remove the oil. Crude oil was centrifuged to remove most of the fish particles and water, polished in a high speed centrifuge to remove the final traces of water, chilled to 5°C, and filtered to remove stearines. The result was light cold pressed (LCP) oil.

Bleached light cold pressed (BLCP) oil was made by bleaching LCP. Bleaching was accomplished by heating LCP to 82°C, adding acid-activated bleaching clay (Fuller's earth), holding temperature for 30 min, then filtering to remove the spent bleaching clay.

Refined light cold pressed (RLCP) oil was made by refining LCP. Sodium hydroxide solution (17-20 Baume; 13-17 g NaOH/100mL; 3.1-4.2 Normal NaOH) (8) was added to the heated (65°C) oil. The mixture was centrifuged to remove soaps, and washed. When RLCP was bleached (as described above for BLCP), the product was called Specially Processed Marine Oil (SPMO, The Zapata Haynie Corporation trademark).

To produce deodorized and stabilized SPMO (SPMO-D&S), the SPMO was steam deodorized/steam stripped in a laboratory glass apparatus by drawing approximately a 5 mm Hg vacuum on the oil. The sample was then heated to 100°C, and steam was added at three to five percent per hour for three hours. After cooling under vacuum, an antioxidant was added (usually vitamin E, but Eastman Kodak Tenox 20 was used in this sample to provide 200 mg/kg tert-butylhydroquinone) to yield SPMO-D&S.

*Sample preparation for vitamin analysis.* The method of Zonta and Stancher (9), as modified by Stancher and Zonta (10), was the basis for preparing samples for vitamin analysis. Approximately 10-g samples of the oils were used for the digestion/saponification of triplicate

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samples of each oil. To each sample was added 0.5 g ascorbic acid, 25 mL distilled water, 50 mL absolute ethanol, and 25 mL of a potassium hydroxide solution (100 g KOH:100 mL distilled water). The flask was sealed with parafilm and shaken gently (150 rpm) overnight in a shaking incubator at room temperature. After centrifuging the sample at 2000 × *g* for 15 min, the supernatant was decanted into an Erlenmeyer flask and extracted with 200 mL of pentane (9). The extract was filtered through anhydrous sodium sulfate, evaporated to dryness under nitrogen, and resuspended in 2-5 mL of high performance liquid chromatography (HPLC)-grade methanol. Final preparation involved filtration through Whatman #1 filter paper and a 0.45 micron syringe-filter. The solution was brought to a known volume (usually 2-5 mL) with methanol for injection into the HPLC.

*HPLC equipment and conditions.* A Beckman System Gold unit, consisting of a #166 ultraviolet (UV) detector, #126 solvent module, and NEC 8300 system controller, was used. Two columns, in series, were utilized (9). One was a Perkin-Elmer (Norwalk, CT) analytical HC ODS/PAH (0.26 cm × 25 cm) column; the other was a Supelco (Bellefonte, PA) 5 micron, 4.6 mm × 15 cm LC-18 column. Both columns were reverse-phase C-18 columns. Flow rate through the columns was 1.50 mL/min, with pressures ranging from 1.5 to 2.2 kpsi. Chromatography was done at ambient temperature.

Table 1 summarizes the solvent system and wavelength changes utilized for chromatography. The solvent system consisted of a basic mixture of HPLC-grade (Fisher Scientific, Cincinnati, OH) methanol and acetonitrile (20:80 v/v). Varying levels of water were added to this basic mixture to facilitate the separation of compounds (9). Wavelengths were also changed to allow maximum peak heights for each of the compounds of interest. Changes in both the wavelength and the percent water in the solvent were made shortly after the eluation of the previous vitamin. For example, the change from 15% water to eight percent water occurred approximately two minutes after elution of vitamin A.

**TABLE 1**

**HPLC Conditions Utilized for the Analysis of Fat-Soluble Vitamins in Menhaden Fish Oil Samples**

| Vitamin | Wavelength (nm) | $H_2O$ in solvent[a] (%) |
|---|---|---|
| A | 325 | 15 |
| $D_3$ | 255 | 8 |
| E | 290 | 8 |

[a] Percent water added to the basic solvent of MeOH:Acetonitrile (20:80 v/v).

Standards consisted of a mixture of vitamins A (all-*trans* retinol and 13-*cis* retinol), E (dl-*alpha*-tocopherol), and $D_3$ (all from Sigma-Aldrich, Inc., St. Louis, MO). Individual standards for each vitamin were also used. Concentrations of the stock solutions (all in methanol) were: vitamin A, 1 mg/100 mL; vitamin $D_3$, 10mg/100 mL; and vitamin E, 50 mg/100 mL. Dilutions were made as needed.

Internal standards were used to identify and quantitate peaks. An 800 μL aliquot of the mixed standard stock solution was added to each oil sample prior to the digestion/saponification step during sample preparation. Quantitation of each vitamin was based on area under the curve, estimated from the product of peak height and width at half height.

## RESULTS AND DISCUSSION

Vitamins eluted from the HPLC column in the following order: vitamin A, vitamin $D_3$ and vitamin E. Times of elution were approximately 7 min for vitamin A, 23 min for vitamin $D_3$ and 34 min for vitamin E.

The fat-soluble vitamin contents of the various menhaden fish oils are shown in Table 2. The bleaching process greatly reduced the fat-soluble vitamin content of the LCP (Table 2). BLCP has less than one-third as much retinol as the LCP. The vitamin D content in BLCP was reduced by approximately one-fifth. If is probable that the vitamins lost from the fish oil were adsorbed to the Fuller's earth.

Alkali refining of the fish oil caused some loss of the fat-soluble vitamins except for vitamin E (Table 2). RLCP had vitamin A and vitamin $D_3$ levels which were approximately 10% lower than the LCP. Vitamin E levels were comparable in LCP and RLCP.

To produce SPMO, RLCP underwent bleaching with Fuller's earth. This treatment caused decreases in fat-soluble vitamin levels similar to those between LCP and BLCP. Vitamin A levels in SPMO were only about 25% the values found in RLCP. Vitamin $D_3$ and E levels were approximately 70% as high in SPMO as those in RLCP.

Steam treatment of fish oil had the most negative effect on the vitamin $D_3$ content. SPMO-D&S had only about one-third as much vitamin $D_3$ as SPMO. This treatment decreased the vitamin E levels by about one-fourth, but had relatively little effect on the vitamin A content.

Processing the fish oil caused substantial loss of all the fat-soluble vitamins. The vitamin A content in SPMO-D&S was less than one-fifth of that found in LCP. Vitamin $D_3$ in SPMO-D&S was also about one-fifth the level in LCP. Vitamin E was affected the least by processing, with levels of about one-half those found in LCP.

**TABLE 2**

**Vitamin Content of Various Menhaden Fish Oils as Affected by Processing**

| | Fish oil (μg vitamin/g oil) | | | | | |
|---|---|---|---|---|---|---|
| Vitamin | LCP | BLCP | RLCP | SPMO | SPMO-D&S | SEM[a] |
| A | 6.19 | 1.78 | 5.74 | 1.19 | 0.95 | ± 0.18 |
| $D_3$ | 26.98 | 17.41 | 25.13 | 17.36 | 5.32 | ± 0.55 |
| E | 116.94 | 85.91 | 117.46 | 86.03 | 62.53 | ± 9.2 |

[a] SEM, standard error of the means, pooled for all samples.

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## REFERENCES

1. Herold, P.M., and J.E. Kinsella, *Am. J. Clin. Nutr. 43*:566 (1986).
2. Herbert, K.E., and E.D. Wills, *Biochem. Soc. Trans. 51*:410 (1987).
3. Hu, E-M., E.N. Frankel, B.E. Leibovitz and A.L. Tappel, *J. Nutr. 111*:1574 (1989).
4. Ackman, R.G., *Food Tech. 42 (5)*:151 (1988).
5. Bimbo, A.P., in *The Seafood Industry*, edited by R.E. Martin, and G.G. Flick, Van Nostrand Reinhold, New York, NY, 1990 (in press).
6. Bimbo, A.P., in *New Technologies for Value Added Products from Protein and Co-products*, edited by L.A. Johnson, AOCS, Champaign, IL, 1989.
7. Bimbo, A.P., *J. Am. Oil Chem. Soc. 64*:706 (1987).
8. Young, F.V.K., International Association of Fish Meal Manufacturers, Fish Oil Bulletin no. 17, *Refining and Hydrogenation of Fish Oil*, Herefordshire, United Kingdom, 1985.
9. Zonta, F., and B. Stancher, *J. Chromatogr. 246*:105 (1982).
10. Stancher, B., and F. Zonta, *Ibid. 256*:93 (1983).

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