

## Stability of Omega-3 Fatty Acids in Fortified Surimi Seafoods during Chilled Storage

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Physical, chemical, and sensory properties of cooked surimi seafood gels (crab analogue) fortified with omega-3 fatty acids (FA) were monitored during chilled storage. Three sources of stabilized omega-3 FA (fish oil concentrate, menhaden oil, and a purified marine oil) were each incorporated into gels to an omega-3 FA content of 1.5 or 2.5%, w/w. Omega-3 FA stability, development of off-flavors, and changes in color and texture were monitored during chilled storage for 2 months. Gels with fish oil concentrate developed fishy flavor and aroma within 30 days and were eliminated from the study. Gels containing menhaden oil and purified marine oil exhibited little change in sensory properties or oxidation products throughout 2 months of storage. Relative polyene index values (ratio of polyunsaturated to saturated fatty acids) indicated that the omega-3 FA were stable at both levels of addition. Omega-3 fortified gels were whiter than control gels, and gel texture was modified when menhaden and purified oils were added but not significantly affected by the level of omega-3 addition.

**KEYWORDS:** Omega-3; surimi; lipid stability; chilled storage

### INTRODUCTION

Consumption of polyunsaturated fatty acids (PUFA) of the omega-3 series, especially eicosapentenoic acid (EPA) and docosahexaenoic acid (DHA), exerts a strong positive influence on decreasing the risk factors associated with the occurrence of several degenerative diseases including cancer, cardiovascular diseases, rheumatoid arthritis, and other inflammatory conditions (1, 2). As the major sources of omega-3 fatty acids (FA) are fish and other marine-derived food products, the daily dietary intake of omega-3 FA in some populations may be extremely low. The use of fish oils in food fortification has been limited due to the greater tendency of long-chain fatty acids to oxidize, resulting in off-flavors and aromas and, consequently, substantial reduction in the shelf life of products. Despite this concern, increased demand for omega-3 FA in the food supply coupled with the introduction of more refined and stabilized fish oils has generated research efforts to fortify a wider variety of food products with omega-3 FA. These include such products as bread, milk, ice cream, and other foods not usually associated with marine origins (3–6). Successful fortification of such foods with omega-3 FA has ranged from 0.2 to 1.8%, depending on the kind of food and the presence or absence of other flavor-masking components (4). For example, in bread, the maximum acceptable level of omega-3 fortification depends on the formulation, particularly the type of flour, as there are major differences in the flavor profile of whole wheat flour versus

white flour (5). Acceptance of omega-3-fortified products also depends on the form of PUFA added and the processing variations. Ice creams to which omega-3 FA were added at concentrations of 250 mg/100 g serving exhibited negligible fish oil flavors using liquid refined fish oil or microencapsulated omega-3 FA from microalgae. However, fishy flavors and aromas were noted in ice creams using other sources such as a microencapsulated fish oil powder and cyclodextrin-complexed fish oil (7).

Because surimi-seafoods are associated with marine sources, are formulated foods, and enjoy wide acceptance in most developed nations, they have become a logical vehicle for increasing the consumption of omega-3 FA without the need for dietary supplements in pill or capsule form (8).

The objectives of this research were to fortify a surimi-based crab analogue to an omega-3 content equivalent to or greater than that of natural salmon fillets (~1.5%) (9, 10) such that product quality and nutritional value were not compromised during a period of chilled storage representative of commercial marketing practices.

### MATERIALS AND METHODS

Frozen surimi commercially prepared from Alaska pollock (*Theragra chalcogramma*) containing 4% sorbitol, 4% sucrose, 0.15% sodium tripolyphosphate, and 0.15% tetrasodium pyrophosphate was obtained from Trident Seafoods Corp. (Seattle, WA). Frozen blocks of surimi (10 kg each) that had been held at  $-20^{\circ}\text{C}$  were impact-shattered, portioned into 1 kg units, vacuum-packaged in oxygen-impermeable bags (CN-590 cook-in material bags, Cryovac, Duncan, SC), and stored at  $-20^{\circ}\text{C}$  until needed. The moisture content of surimi was determined to be 76.4% (11).

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**Table 1.** Summary of Fatty Acid Composition of Fish Oil Concentrate, Menhaden Oil, and Purified Marine Oil Expressed as Percentage Composition of Total Fatty Acids Identified

major classes of fatty acids	fish oil concentrate	menhaden oil	purified marine oil
saturates	2.0	41.5	38.5
monoenes	6.1	34.0	31.4
PUFA <sup>a</sup>	91.9	24.5	30.1
<i>n</i> -3 PUFA <sup>b</sup>	89.5	21.5	26.8

<sup>a</sup> PUFA, total polyunsaturated fatty acids. <sup>b</sup> Total *n*-3 polyunsaturated fatty acids.

**Preparation of Surimi Paste.** Control gels to which no oil was added consisted of added salt and water to levels of 1.5 and 75% (w/w), respectively. Other additives were 8% potato starch (Pencook 20, Penford Food Ingredients Co., Englewood, NJ); 2% egg white powder (McWaldbaum, Wakefield, NE); 1.0% sorbitol (sorbitol 70/20, Roquette America, Inc., Keokuk, IA); 1.0% sucrose; and 3.7% (w/w) of crab flavor and aroma additives supplied by Activ International (Seattle, WA). This formulation closely imitates that of most commercial crab analogue products being marketed.

In treatments containing added omega-3 FA oils, the amount of water was substituted by oil. Three sources of omega-3 FA were used: a fish oil concentrate (20/50 TG, Ocean Nutrition Canada, Ltd., Bedford, NS, Canada), refined menhaden oil (Omega Pure, Omega Protein, Reedsville, VA), and a purified marine oil (ROPUFA 30 *n*-3 Food Oil, Roche Lipid Technologies, Derbyshire, DE). The fish oil concentrate (**Table 1**) contained 89.5% omega-3 FA and mixed tocopherols; the refined menhaden oil contained 26.5% omega-3 FA and a combination of mixed tocopherols and tertiary butylhydroxyquinone (TBHQ); and the purified marine oil contained 26.8% omega-3 FA and mixed tocopherols, ascorbyl palmitate, and a rosemary extract. These oils were added at levels of either 1.5 or 2.5% (w/w). As each of the fish oils contained different concentrations of omega-3 fatty acids, each level of addition was adjusted to result in the same level (i.e., 1.5 or 2.5%) of omega-3 FA added to each gel. The lower level is roughly equivalent to that naturally occurring in salmon flesh (9, 10), and the higher level was designed to test the maximum levels achievable without a loss of the physical and sensory characteristics of the surimi gels in comparison to control.

Prepared surimi pastes were extruded into stainless steel tubes (22.7 cm length, 1.9 cm i.d.) using a sausage stuffer (The Sausage Maker, Buffalo, NY). A two-step thermal treatment was used. Samples were first cooked in a 90 °C bath for 6 min to allow gelation of surimi, roughly simulating rapid cooking achieved by continuous sheet cooking in industry. Samples were held at this temperature for 8 min, removed from the tubes, and placed in a double vacuum plastic bag with low permeability to oxygen (Cryovac, Duncan, SC). A second heat treatment in an 85 °C bath for 15 min was then applied in simulation of industrial pasteurization practices (12). Samples were then immediately cooled in an ice/water slush and subsequently held below 4 °C for up to 2 months. Chemical, physical, and sensory analyses were conducted initially (after overnight storage) and at 1 month intervals.

**Sensory Evaluation.** A six-member trained sensory panel performed descriptive sensory (flavor, odor) analyses. Aroma, flavor, and after-taste notes were determined using a 14-point scale: not detectable (0–2), threshold (2–5), slight (6–9), moderate (10–13), and strong (14). The panel was initially trained (13, 14) to evaluate flavor changes in fish gels and was retrained in this study to score crab product attributes with emphasis on recognizing fishy odor and aroma as these are the two negative sensory attributes most often associated with the addition of omega-3 FA. Control gels containing no added oil and stored at –50 °C were used for reference. Each sensory property was expressed as the average of the data from all panelists.

**Lipid Stability.** Production of *lipid oxidation volatiles* was determined by the manual solid-phase microextraction (SPME) technique for low molecular weight oxidation compounds (15). A SPME extractor containing a 75 μm filter of Carboxen polydimethylsiloxane (Supelco, Bellefonte, PA) was used. Two grams of chopped gel was placed in a

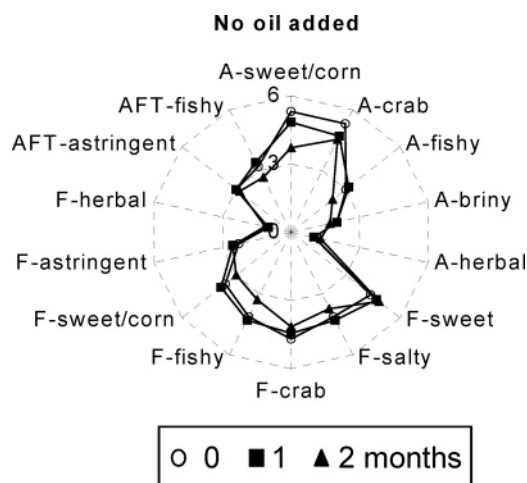
12 mL vial (Tekmar Dohrmann Co., Cincinnati, OH), and the vial was flushed with nitrogen, closed with a headspace clamp, and kept in chilled storage at 4 °C for up to 2 months. Headspace was sampled at 0, 1, and 2 months. Oxidation volatiles present in surimi gels were extracted onto SPME fibers by heating the vials at 60 °C for 15 min with the manual SPME apparatus in the injection mode. Desorption of volatiles from the fiber onto the GC column occurred by heating the SPME apparatus in the injection mode of the GC injection port for 2 min at 180 °C with a carrier gas flow rate of 2.0 mL/min. An HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Hewlett-Packard, Palo Alto, CA) was used for separation. Volatiles were identified by comparison to authentic standards followed by confirmation with a GC-MS system consisting of an HP 5890 II GC and an HP 5972 mass selective detector (Hewlett-Packard, Avondale, PA). The GC oven temperature was cryogenically cooled and programmed from –20 to 160 °C at 15 °C/min with initial and final hold times of 4.5 and 1 min, respectively. The oven temperature was programmed for an increase of 40 °C/min from 160 to 220 °C and a final hold time of 1.5 min. The carrier gas was electronically controlled at a linear velocity of 1.5 mL/min. The ionization voltage for the mass spectrometer was 70 eV. The mass scanning range was 35–350 kDa. Two replications were performed.

The *polyene index* [ratio of polyunsaturated (PUFA) to saturated (SAT) fatty acids] was calculated from the gas chromatographic data. As DHA (C22:6) represents the most highly unsaturated and most labile fatty acid present in fish oils, the loss of DHA relative to palmitic acid (C16:0) is often used as a sensitive index indicative of the oxidation of PUFA and was therefore used in the present study (16, 17). It was expressed as the percentage of polyene index relative to the polyene index of the raw oil.

To determine the *fatty acid composition* of each oil and of omega-3 enriched gels, total crude lipids were extracted according to a modification of the Bligh and Dyer (18) procedure in which 0.001 g/L of BHT was added to a chloroform/methanol solvent system (1:2), followed by extraction, cleanup, and subsequent drying of lipids. The fatty acid methyl esters (FAME) were prepared by the addition of 2 mL of boron trifluoride per 25 mg of lipid. Crude FAME were washed with distilled water, extracted twice with 2 mL of hexane, and dried under nitrogen at 35 °C (19). Crude FAME were purified by thin-layer chromatography using a solvent elution system consisting of petroleum ether/diethyl ether (95:5), followed by drying under nitrogen. Purified FAME were redissolved in iso-octane for injection. The FAME were analyzed on an HP model 5890A GC (Avondale, PA), equipped with a flame ionization detector, an HP 3393 integrator, and a Dell computer, model D1028L (Round Rock, TX) for data storage and handling. The GC contained a DB 225, 30 m × 0.25 mm fused silica column (J&W Scientific Co., Folsom, CA) that was temperature programmed from 140 to 200 °C at a rate of 1.7 °C/min with both injector and detector temperatures set at 260 °C. Identification of FAME was based on comparisons of retention times of test samples to methyl ester standards (462 and 68D, NuChek Prep, Elysian, MN) using C21:0 as the internal standard. Absolute response factors were calculated for each identified fatty acid peak using the normalization technique described by Sampugna et al. (20). Fatty acid composition was expressed as weight percent of total FAME and was determined in duplicate.

**Gel Color Analysis.** Color differences between treatment gels and the control were determined by reflectance on a Chroma Meter CR 300 (Minolta Camera Co., Ltd., Osaka, Japan) using the CIE Lab scale (D65/10°), where *L*\* (black 0 to light 100), *a*\* (red 60 to green –60), and *b*\* (yellow 60 to blue –60) were used to measure lightness, redness, and yellowness. Whiteness index was defined as follows:  $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ . The result was expressed as the average of six samples.

**Gel Fracture Properties.** Gel samples were tempered to ambient temperature (20 °C) prior to analysis. Gels were cut into 29-mm-long cylinders, each end of which was glued to plastic disks using cyanoacrylate glue. The cylinders were ground on a Precision Machine (model GC-TG92 US, Gel Consultants, Inc., Raleigh, NC) and formed into capstan shapes with a center diameter of 10 mm. Gels were vertically mounted and twisted to fracture at 2.5 rpm on a Hamann



**Figure 1.** Change in sensory attributes of surimi gels containing no added omega-3 fatty acids. A, aroma; F, flavor; AFT, aftertaste.

Torsion Gelometer (Gel Consultants Inc., Raleigh NC) to determine stress (strength) and strain (deformability) at fracture displacements as described by Diehl et al. (21). Standard deviations on measurements among replicates (six samples) were <10%.

**Statistical Analysis.** One-way analysis of variance (ANOVA) was carried out using the SPSS system (SPSS Inc., Chicago, IL). Significant differences between pairs were determined by using the Bonferroni test. In the case of sensorial test, significant difference between means was resolved by means of confidence intervals using nonparametric statistics by the Kruskal–Wallis test using the SPSS system (SPSS Inc.). Level of significance was set at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

**Sensory Testing.** Food product palatability is one of the main sensory characteristics limiting the level of fortification with omega-3. Results of this study showed that 2.5%  $n-3$  was the highest level of omega-3 FA that was acceptable for the sensory panel (taste and texture) (data not shown). The faint and fish-like odor was masked by the added crab flavor aroma extracts, allowing the use of a significant level (2.5%) of fish oil. These findings agree with those of Kolanowski et al. (4), who demonstrated that high flavor intensity products are more effective at masking the slightly fishy taste and odor associated with the addition of omega-3 FA.

**Figure 1** shows the profile of the descriptive sensory attributes of control surimi gels with no oil added. However, it should be noted that the additions of the crab flavor resulted in noticeable crab aroma–flavor and sweetness notes in the crab analogues. Crab gels formulated with fish oil concentrate (**Figures 2 and 3**) contained significantly higher intensity of fishy attributes including taste, aftertaste (AFT), and aroma than present within the control (**Figure 1**). For treatments containing fish oil concentrate, the sensory attributes reached the rejection level of off-flavors and aroma at both levels of addition (i.e., 1.5 and 2.5%) after 1 month of storage. Thus, treatments containing fish oil concentrate were not evaluated for further sensory analyses. These results show that although the strong crab flavor was effective in masking moderate fishy taste and aroma attributes, the fish oil concentrate contained an especially high concentration of fishy sensory notes that would not allow its use in a typical processed seafood product, such as a crab-flavored analogue.

Treatments containing menhaden oil and purified marine oil (**Figures 2 and 3**) showed sensory profiles more similar to those of the control gel containing no added oil. Even though the sweet/corn flavor that was observed in the control samples was

not present in treatments containing menhaden and purified marine oils, the added crab flavor and aroma were effective in masking trace amounts of off-flavors that may have resulted from addition of the omega-3 fish oils. Other studies in which menhaden oil was used in cookie formulations showed that when the cookies were stored at room temperature, fish oil-containing cookies reached the rejection level of off-flavor intensity (mildly oxidized off-flavor) at 10 days, whereas treatments containing added ascorbic acid reached an additional 7 days of shelf life (6). The author explained that the combination of food ingredients and an oven-baking process caused menhaden oil to be more vulnerable to oxidative degradation.

**Lipid Stability.** Formation of volatile compounds was similar in all samples. Among 10–15 peaks detected, the major compounds positively identified were acetaldehyde, ethanol, dimethyl disulfide, ethyl acetate, butanediol, and hexanoic acid. As volatile concentrations did not increase significantly during storage, whereas ethanol was used as a carrier for the crab flavors added, it was concluded that the extent of oxidation during the 2 month storage period was not a significant factor in decreasing sensory attributes. Immediately following elaboration of the gels, minor concentrations of hexanal was also observed. However, as hexanal is a major oxidation product of omega-6 oxidation and appeared as a minor peak in control samples and decreased with storage, it was concluded that it too was not a major contributing factor to changes in sensory profiles or in lipid stability.

The processing of fish oils to remove unwanted odors and off-flavors has also been improved considerably, with some studies (6) showing that during the refining of fish oil, the process removes almost all compounds related to fishy odors. However, during storage, additional odorous compounds such as pentylfuran and aldehydes can be generated. These types of compounds cause green and beany odors, whereas the presence of ketones creates metallic-type off-flavors. The presence of decatrienal and heptenal has been shown to impart fishy odor to foods. The production of volatiles is also influenced by the degree of unsaturation of the oil. One such study (22) identified unsaturated aldehydes and ketones containing two or three double bonds that were highly correlated with the loss of DHA during storage of fish oil. High correlation between both hexanal and pentanal levels as determined by SPME-GC and lipid oxidation as measured by 2-thiobarbituric reactive have been observed in other oxidation studies (15). Studies of beef gels stored under chilled conditions found increased concentrations of total aldehydes, hydrocarbons, and alcohols due to storage time at 3 °C, whereas total ketones varied due to time only (23).

Thus, the combined effects of short storage time in this study (i.e., 2 months), chilled storage conditions, and the presence of volatile oxidation products below the level of detection may all have contributed to the absence of significant oxidation products being measured in this study. The recent availability of more highly stabilized fish oils containing a variety of antioxidants may also have played a significant role in reducing the degree of oxidation and the lack of measurable oxidation products. All of the oils used in this study had been stabilized with a combination of commercial antioxidants and natural antioxidants that may well have contributed to the absence of off-flavors and odors observed during chilled storage.

**Fatty Acid Composition.** **Figure 4** shows a comparison of the change in the polyene index in relationship to the composition of the raw oils from each one of the three sources of added fish oil. Due to the heat treatment, there was a decrease in



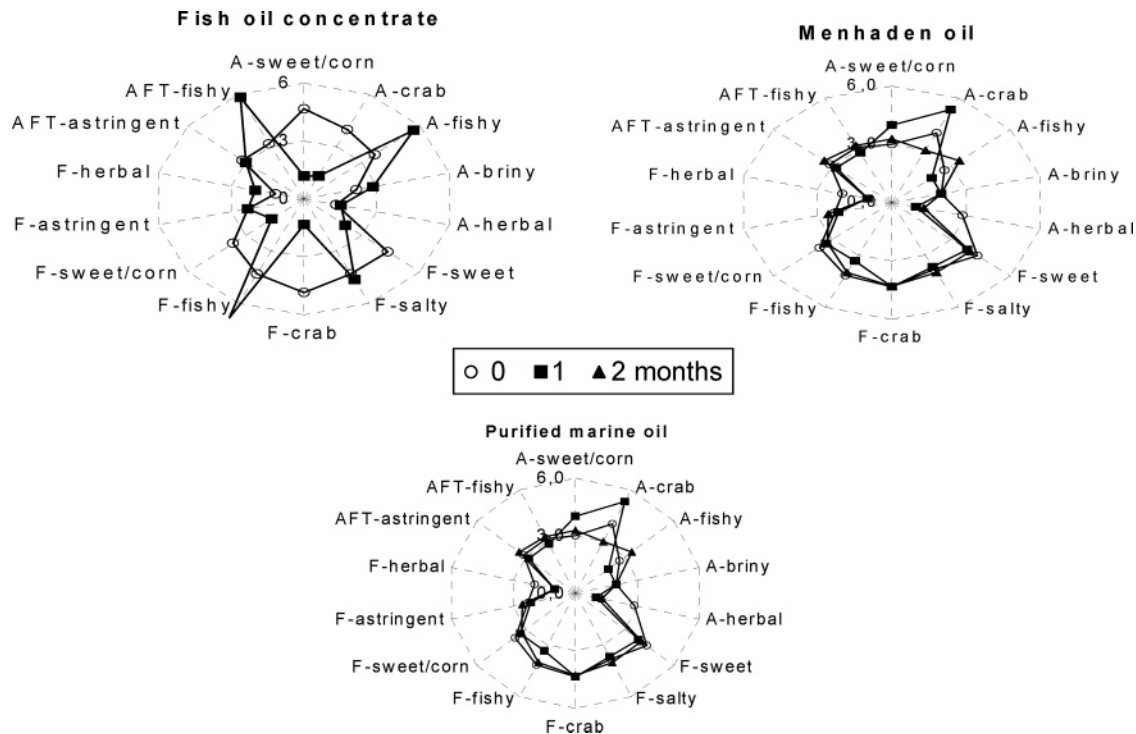


Figure 2. Change in sensory attributes of surimi gels fortified at 1.5% omega-3. A, aroma; F, flavor; AFT, aftertaste.

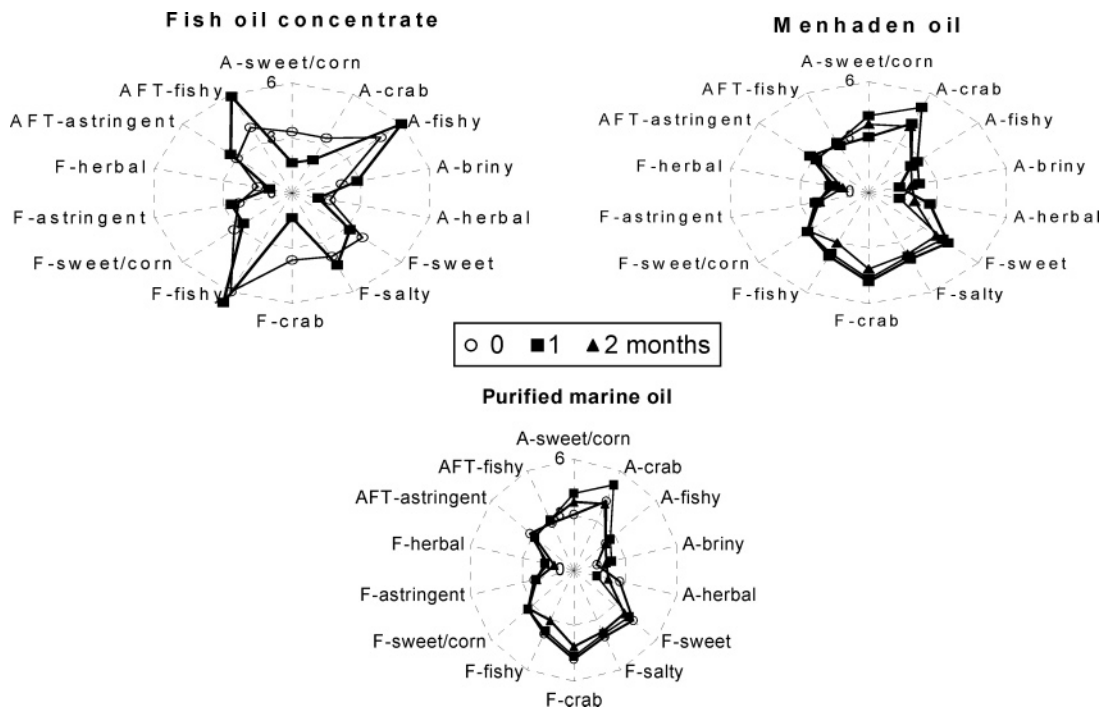
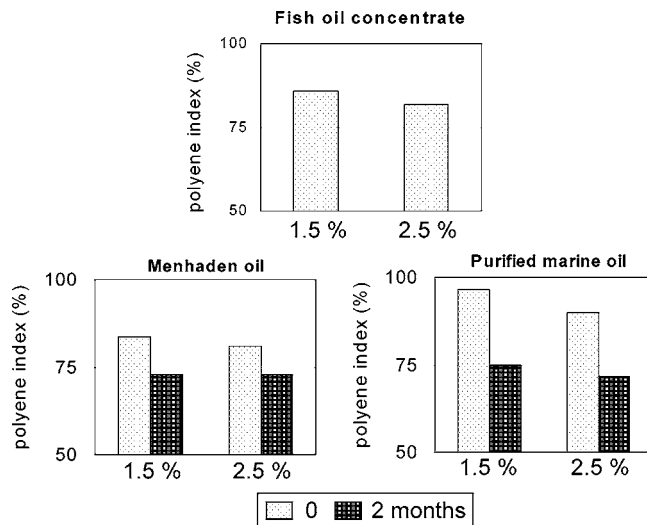


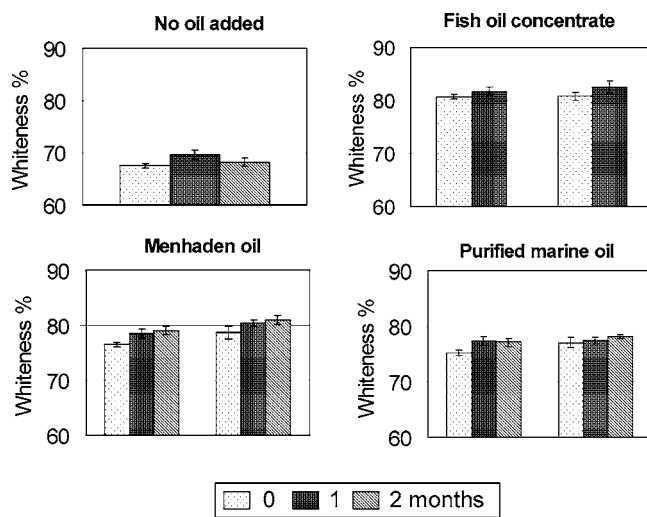
Figure 3. Change in sensory attributes of surimi gels fortified at 2.5% omega-3. A, aroma; F, flavor; AFT, aftertaste.

polyene index values at different percentages depending on type of oil added. A 15–20% decrease occurred in samples made with fish oil concentrate and also menhaden oil, and only 5–10% occurred in purified marine oil. In all cases, there was a tendency of higher losses when using higher amounts of omega-3 FA, although none of the losses between treatments were significant. However, after 2 months of chilled storage, the percentage of loss was similar in menhaden and purified marine oil samples (~25–30%). Therefore, with the exception of oils lost during the initial cooking operations, all of the treatments maintained ~75% of the polyene index of the raw oil at the end of the storage.

Ghazala et al. (24) studied the stability of fatty acids after pasteurization and found that more EPA and DHA are retained when the pasteurization process is done at a low temperature (65 °C) compared to a higher temperature. Differences in the inherent composition of the commercial oils (Table 1) and their antioxidants may also have played a role. For example, the fish oil concentrate contained the highest level of omega-3 FA and was the only oil in which only mixed tocopherols were used as the only type of antioxidant added. Although tocopherols are regarded as excellent antioxidants, their effectiveness as the sole antioxidant in fish oils has not been equivalent to that of combinations of natural and synthetic antioxidants, especially



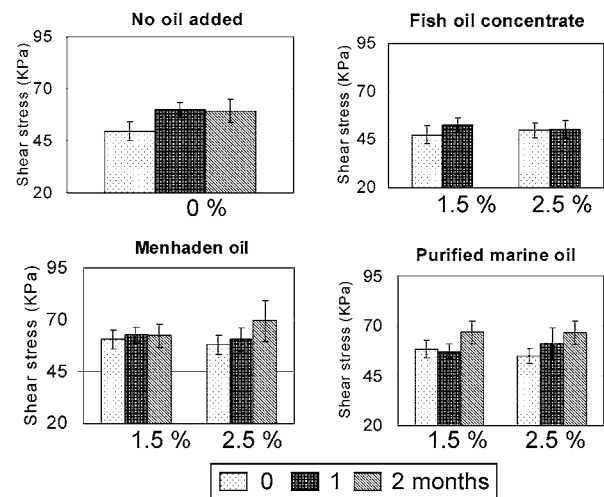
**Figure 4.** Change in polyene index (ratio of C22:6 to C16:0) of surimi gels after cooking (lightly shaded bars) and after 2 months of chilled storage (heavily shaded bars).



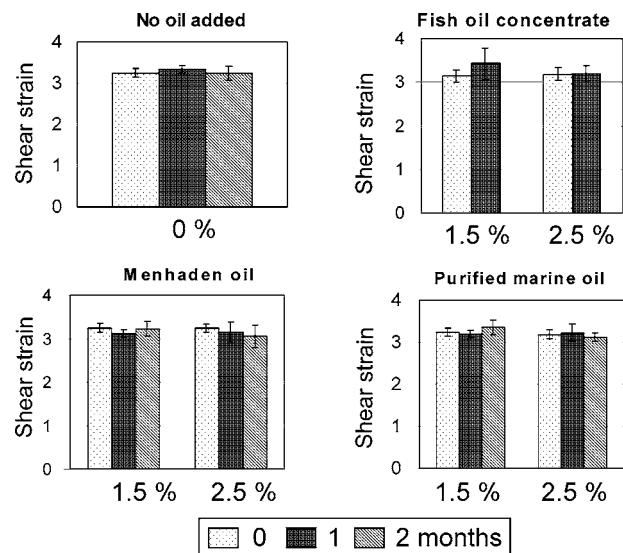
**Figure 5.** Change in whiteness of surimi gels containing the different oils and levels of omega-3. Error bars represent the standard deviation of six samples.

TBHQ. Both the menhaden oil and the purified fish oil contained lower levels of omega-3 FA and a variety of natural and synthetic antioxidants that could have contributed to a greater degree of stability observed in these two oils compared to the fish oil concentrate. The purified marine oil contained added rosemary, which may have contributed to the higher protective effect in this relatively short term study, although its effectiveness in long-term study has been questioned. Similar studies (25) have reported that the addition of rosemary extract may offer limited extension of storage stability because of its involvement in the initial free radical reactions.

**Gel Color.** Only minor changes in color occurred during chilled storage (Figure 5). Initially the control gel (no oil added) was less opaque and significantly ( $p \leq 0.05$ ) lower in whiteness index than those fortified with oils. The increased whiteness can be attributed to the increase in lightness ( $L^*$  values) caused by the dispersion of light resulting from the presence of emulsified oil droplets. Because whiteness of color is a desirable attribute in surimi-seafood products, the addition of oil only enhanced the quality of the product. During chilled storage, there was a tendency toward increased whiteness with increased



**Figure 6.** Change in shear stress values of surimi gels containing the different oils and levels of omega-3. Error bars represent the standard deviation of six samples.



**Figure 7.** Change in shear strain values of surimi gels containing the different oils and levels of omega-3. Error bars represent the standard deviation of six samples.

storage time, with most of the samples showing significant ( $p \leq 0.05$ ) increases after the first month of chilled storage.

**Gel Fracture Properties.** There was a significant increase in shear stress of gels containing menhaden oil and purified marine oil at both omega-3 concentrations at the beginning of storage in comparison with control with no oil added. This was expected because of the added oil substituted for the same amount of water used in the formulation (Figure 6). No increase in shear stress occurred in the case of fish oil concentrate because it contained the highest level of omega-3 FA and required the smallest amount of oil (~2–3%) to obtain the desired level. Although oil as an ingredient can modify the characteristics of the gels, no major modifications were obtained because the formulation contained starch and egg white. These results agree with a similar study carried out by Pérez-Mateos et al. (26) in which increased breaking force and hardness were observed in heat-induced mackerel gels containing omega-3 oil added at the 2.5% level compared to gels containing no added oil.

Stress (strength) increased in gels with no oil added during the first month of storage ( $p \leq 0.05$ ), as would be expected

due to bond realignment in the first weeks (**Figure 6**), and a similar tendency to increase with storage time was observed in samples containing added oil, although not significant in that case.

Shear strain values (**Figure 7**) did not decrease with the addition of oil, and no significant differences were found due to the type of oil used because the water content was adjusted depending on the amount of oil added. Shear strain values remained stable throughout the storage in all of the samples.

**Conclusions.** Fortification of surimi-based crab analogue products to contain omega-3 FA levels equal to or exceeding that of natural seafood products appears to be quite feasible. The sensory and analytical data showed that the type of fish oil used and its antioxidant system can be critical to avoiding off-flavors and aromas that can distract from consumer acceptance of such products. Fish oils can be added to attain levels of 2.5% omega-3 FA without adversely affecting the taste, stability, color, or texture of crab-flavored seafood gels.

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