1992-1

IAFMM

International Association of Fish Meal Manufacturers

RELEASED
PARTLY RELEASED
CONFIDENTIAL ✓
DATE: 8 Apr 92

CHEMICAL COMPOSITION AND STABILITY OF FISH OIL

Sponsored by IAFMM

Final Report, April 10, 1991

(Part I)

by

HERBERT O. HULTIN *and* SAJIDA S. JAFAR

Massachusetts Agricultural Experiment Station
Department of Food Science
University of Massachusetts/Amherst
Marine Foods Laboratory, Marine Station
Gloucester, MA 01930

RESEARCH REPORT NUMBER: 1992-1

Dr. S. Barlow
Intern. Assn. of Fish Meal Mfrs
Hoval House, Hutton Lane
Potters Bar
Herts EN6 3AR
Association
2 2 2

STRICTLY CONFIDENTIAL

SUMMARY

1. Eight different fish oils (three of which were supplied with and without antioxidants) were evaluated chemically and by sensory methods for their quality and stability. A wide variability was observed in initial quality and various measurements of stability.

2. There appeared to be little relation between the chemical composition of the oils and their stability as measured by the length of the induction period at 70°C determined by measurement of anisidine values. There were many undefined variables in the samples; this made it difficult to draw conclusions.

3. Results indicated that it is possible to prepare very high quality oils from fish.

OBJECTIVES

The objectives of this study were:

1. To determine the chemical composition of fish oils manufactured in various countries, to estimate the sensory quality and stability of the oils and to relate, if possible, the chemical composition to the stability of the oils. IAFMM was to arrange to have the fish oils supplied. (Part I of Report)

2. To incorporate fish oil into food products while obtaining an acceptable shelf-life. Originally two products were to be evaluated, mayonnaise, an oil-in-water emulsion and margarine, a water-in-oil emulsion. Time limitations and difficulty of preparing the product did not allow us to evaluate fish oils in margarine. Thus, only mayonnaise was evaluated. (Part II of Report)

MATERIALS AND METHODS

Fish oils were supplied to us from Chile, Denmark, Faroe Islands, Iceland, Norway, South Africa, Sweden and the United States. Most of the oils did not have added antioxidants. Three of the eight suppliers provided extra samples with antioxidant mixtures they normally add. These were also evaluated. Additional samples of oil were supplied to us by two companies for the work with mayonnaise.

Chemical Analyses

Fatty acids were measured by gas chromatography after methylation with BF_3 in methanol. The methyl esters were injected into a Varian Model 3700 gas chromatograph equipped with a Hewlett-Packard 3380A integrator. A temperature program was used with an initial temperature of 160°C held for 4 min then increasing to 225°C at a rate of 4°C per minute. The injector and FID detector temperatures were 250°C and 280°C,

respectively. The nitrogen flow rate was 30 ml per min using a Supelco-wax 10 column. The fatty acids were quantitated using an internal standard of the 23:0 fatty acid. Results are reported as mg of fatty acids per 100 mg of oil. For each oil a number of minor peaks were unidentified and are not included in the total.

The Wijs iodine value and free fatty acids were determined according to the AOAC Methods, 28.021 (1980). The amount of free fatty acids were expressed as the 22:6. Tocopherols were determined by normal phase liquid chromatography using the method of Carpenter (JAOCS 56 (1979) 668). Separation was performed on a 4.6 mm x 250 mm Microsorb silica column with 5 u particle size and 100 A pore size (Rainin Co.). The mobile phase was 1.5% isopropanol in hexane with a flow rate of 1 ml per minute. A DuPont module 870 pump was used. Sample injections were made with a 20 ul Reodyne loop injection valve (7010). A Hewlett-Packard 3396 integrator was used. Tocopherols were detected at a wavelength of 295 nm with a Waters model 484 detector. Retinol was determined on the same sample using a detector wavelength of 325 nm (J. Food Sci. 55 (1990) 77).

Iron and copper were determined with a Perkin-Elmer 3030B atomic absorbtion spectrophotometer with a HGA-400 graphite furnace accessory. The oil was used directly without predigestion with pyro coated platform. Iron concentrations were determined at 248.3 nm with a slit width of 0.2 nm. Pretreatment temperature was 1400°C with a ramp time of 10 sec and a hold time of 20 sec. Atomization temperature was 2400°C for 5 sec. Copper was analyzed at 324.8 nm with a slit width of 0.7 nm, a pre-treatment temperature of 1200°C with a ramp time of 12 sec, hold time of 20 sec and atomization temperature of 2300°C for 4 sec.

Carotenoids were determined by dissolving portions of the oil in hexane and measuring absorbtion at 466 nm with a Hitachi Model U-3110 spectrophotometer. Results are expressed as canthaxanthin using an extinction coefficient of 2200.

Tests for oxidation included the anisidine value (IAFMM Fish Oil Bulletin, No. 8, June, 1981), peroxide value as meq per kg oil (Woyewoda et al., Can. Tech. Rep. Fish. Aquat. Sci., No. 1448, p.73, 1986), and thiobarbituric acid reactive substances as mg malonaldehyde per 100g oil (J. Dairy Sci. 34 (1951) 669). Conjugated dienes and trienes were measured at 232 and 270 nm, respectively; data are presented as specific extinction (E) of 1% solution of the oil in isooctane at the indicated wavelength.

Stability Studies

Accelerated stability studies were performed by filling three 50-ml test tubes with 40 ml of oil each. The tubes were placed in a 70° water bath and air was bubbled into each tube at an average rate of 12.1 ml per sec. Samples were taken initially and at various time intervals; anisidine values were measured. The induction period was taken as the period of time indicated by the point where the initial slope of the rate curve intersected the slope of the accelerated oxidation phase.

Longer term stability studies were accomplished by filling 250-ml flasks with 60 ml of oil, covering the flask with parafilm and storing at room temperature. Samples were taken

initially and at selected intervals of time for sensory and chemical tests. The latter included anisidine value, peroxide value, TBA-reactive substances (TBARS) and conjugated dienes and trienes.

Sensory evaluation of the oils were performed using both odour and taste according to the method of the AOCS (1989) with modifications suggested by the Norwegian Herring Oil and Meal Industrial Research Institute. Twenty ml of oil were placed into 250-ml beakers which were then covered with aluminum foil and put into a 50° water bath for 30 min before evaluation. After each evaluation the beakers were returned to the water bath to maintain that temperature. Two references were used. Reference 1 was a freshly refined soy bean oil representing a score of 10, i.e., no off odour or flavour. Reference 2 was a mixture of oxidized fish oil with soy bean oil with a strong oxidized flavour representing a score of 2.

RESULTS

Chemical Composition and Stability of the Fish Oils.

Fish oils were received from 8 producers around the world. Means of shipment were not consistent and may have played some part in initial quality and/or stability. For example one producer sent their sample to IAFMM headquarters in the U.K. which was then sent to us in Gloucester, U.S. For the most part the fish oils did not have protective agents added. However, 3 manufacturers sent samples of stabilized fish oils as well. Thus sample B is the same as sample A except it has added antioxidants, G is the same as F but with antioxidants and I is the same as H but with antioxidants. In addition, fish oil E had citric acid added and fish oil K had vitamins A and D.

The results of the chemical analyses of the fish oils (A-K) and their stabilities are summarized in Tables 1 - 23. The oils are presented in order according to their alphabetical designation, giving first the chemical analysis of the oil and then the results of the stability study. Thus, Table 1 is a chemical analysis of fish oil "A", and Table 2 is work based on its stability. Included in each stability table is the induction period of the oil as measured by anisidine value at 70°C. A summation of the induction periods of each of the fish oils at 70°C is given in Table 24.

One of the purposes of evaluating the several fish oils was to give the individual producers an idea of the composition, quality and storage stability of their product. It is hoped that the information presented is useful in that respect.

Another hoped-for result from the data would be some correlation between chemical composition and potential stability of the oil. This aspect of the study turned out disappointing. The induction period at 70°C as measured by anisidine value was assumed to represent a chemical measurement of the relative stabilities of the oil (Figure 1). No consistent relationship was observed between this value and iodine number (Figure 2), tocopherol content (Figure 3), content of transition metals (Figure 4), totox values (Figure 5), or rate of change of PV (Figure 6). Six of the eight oils had induction periods at 70° that ranged from 5 to 8 hr; 2 of the oils were considerably below this. These latter two oils D and K had high iodine values and their instability may be a

reflection of this high degree of unsaturation. Oil C also had a high iodine value but this might have been counteracted by its low content of transition metals, by far the lowest of all the samples. As expected, the samples which had antioxidants added to them had much longer induction periods; there was very little difference among the 3 stabilized oils in terms of their stability as determined by measurement of induction period.

Sensory evaluation of the oils proved to be a problem. The scale that was used confined the fish oils acceptable as human food to the very upper end of the scale. Although preference studies were not carried out, it was generally agreed among the panel that sensory scores much below 8.0 would not make an acceptable food product. We used reference samples of 10 and 2. The latter was extremely objectionable. Using only a very good reference and an extremely poor one as reference points made it difficult for the panellists to consistently judge the degree of objectionableness of the samples. The panel improved significantly with time, but this aspect remained a problem.

Only three of the oils A, C and E were considered to be acceptable for direct incorporation into human foods (oil B which is the same as A but with antioxidants added was also very good). Two of the oils received were below the second reference point, i.e., below a score of 2.

These results show that high quality fish oils can be produced but that great care must be taken to do so. It would seem useful to evaluate the various processing and refining steps to determine what is necessary to produce high quality fish oils.

Presumably, the initial oil quality was a reflection of the processing procedure. We recognize that this might be an oversimplification. Since there had to be some storage time during the transportation of the oil to our laboratory, stability questions may have had a role in this initial quality of the various oils. One of the high quality oils (oil A) generally had relatively high anisidine values (Figure 5). This did not seem to detract much from its initial quality, and the rate of increase in anisidine was no greater in this oil than in others. Indeed there were low quality oils that did not show a rapid increase of anisidine during room temperature storage. The changes in anisidine values with time at room temperature did not give clearly defined induction periods, contrary to what was observed at 70°C with oxygen bubbled through the oil. This may simply be a reflection that the oxidation at room temperature was not carried out to such a severe extent as at 70°C. It is also possible that relative oxidation rates at 25° and 70° are not consistent among the oil samples.

It was particularly surprising to see the lack of relation between the content of iron and copper and the stability of the oil (Figure 4). The content of iron was extremely low in sample C, and there was no detectable copper. Nevertheless, the stability of this oil was not greater than other samples that had considerably more transition metals. A problem with any evaluation attempted is that many factors are unknown. Thus, it is difficult to pinpoint what is causing deterioration. There are several explanations for the data with the transition metals. One is that an extremely small amount of iron and copper is all that is needed to initiate the oxidation. If this is the case, it would indicate that transition metal-catalysed oxidations can only be controlled by proper chelation techniques. It could also be that sample C was susceptible to a low iron content because

of its high unsaturation. Alternatively, it could mean that mechanisms other than those initiated by transition metals are important under some circumstances. For example, we did not do any studies on the oils in which we attempted to exclude light. It is known that photo-induced oxidations can function via the generation of singlet oxygen. This oxidation mechanism produces different end products than those produced by transition metals and might account for some of the discrepancies seen amongst the data. The reaction could be controlled by differing levels of sensitizer molecules in the different oils. This possibility was not explored.

GENERAL DISCUSSION AND FUTURE CONSIDERATIONS

It is clear that high quality fish oils can be produced. They are, however, unstable. One of the difficulties in determining initial quality of the oils in this present study was the means by which the oils were received at the laboratory. It was not possible to closely control all factors involved during the transportation, and it can only be hoped that the initial quality as received at our laboratory was similar to that when it was produced. For those producers who had low quality oils, it might be useful for them to determine the factors in production and refining which are critical in obtaining the high quality oils achieved by some producers.

We did not make a great deal of progress in relating chemical composition of the oils to their stability. This was partly due to the large number of samples that had to be examined and the relatively limited amount of time, and partly to the fact that there were too many uncontrolled variables with each of the oils. To determine the factors which are important in the susceptibility of a oil to oxidative deterioration, it is necessary to start with a high quality oil and to test the factors in a precise and logical way. Initially it would seem reasonable to change one factor at a time, e.g., transition metal content. When this has been accomplished, then those factors that seem to be important can be evaluated concurrently. Environmental conditions such as temperature or exposure to light may affect the relative stabilizing or de-stabilizing power of the various components.

There appeared to be little correlation between the absolute values of the chemical indices and the quality of the oils. In some cases, changes occurring in peroxide values or anisidine values were useful in evaluating changes; however, the initial value did not appear to be related to the changes observed. When a given oil under a given set of conditions was used, for example in some of the mayonnaise studies, some of these chemical indices appeared to be reasonably good measures of change. This behaviour is well known in lipid chemistry. Oxidative tests are often useful to follow the progress of deterioration, but are not particularly good as absolute measures of quality. Better measurements of quality would be most useful. As was suggested at one of the IAFMM meetings, headspace analysis might be worth consideration for development as an objective measure of oil quality.

In the meantime, we believe that sensory evaluation is the best method to use. We had some problems with the current methodology in using it as a means of determining extent of deterioration. For fish oils to be used as human food, we would recommend that the technique that we used with the mayonnaise work be considered (see Part II).

This was essentially to measure the induction period to when the first off-odour was recognized (taste could also be used). It was assumed that any oxidized odour development is unacceptable. In practice this technique worked well. It is very simple to do and requires only simple standards. There was little member-to-member variation by the sensory panel when this technique was used. It is believed that this is due to the fact that odour and flavour development follow typical oxidation kinetics, i.e., a lag phase. Even with different panel members having different sensitivities to off-odour development, the change goes relatively rapidly once it starts and thus the induction periods observed by different panel members falls within a relatively narrow range.

ACKNOWLEDGMENTS

We thank Stephen D. Kelleher for expert technical advice and assistance and all panel members for their help and cooperation.

FIGURE 1

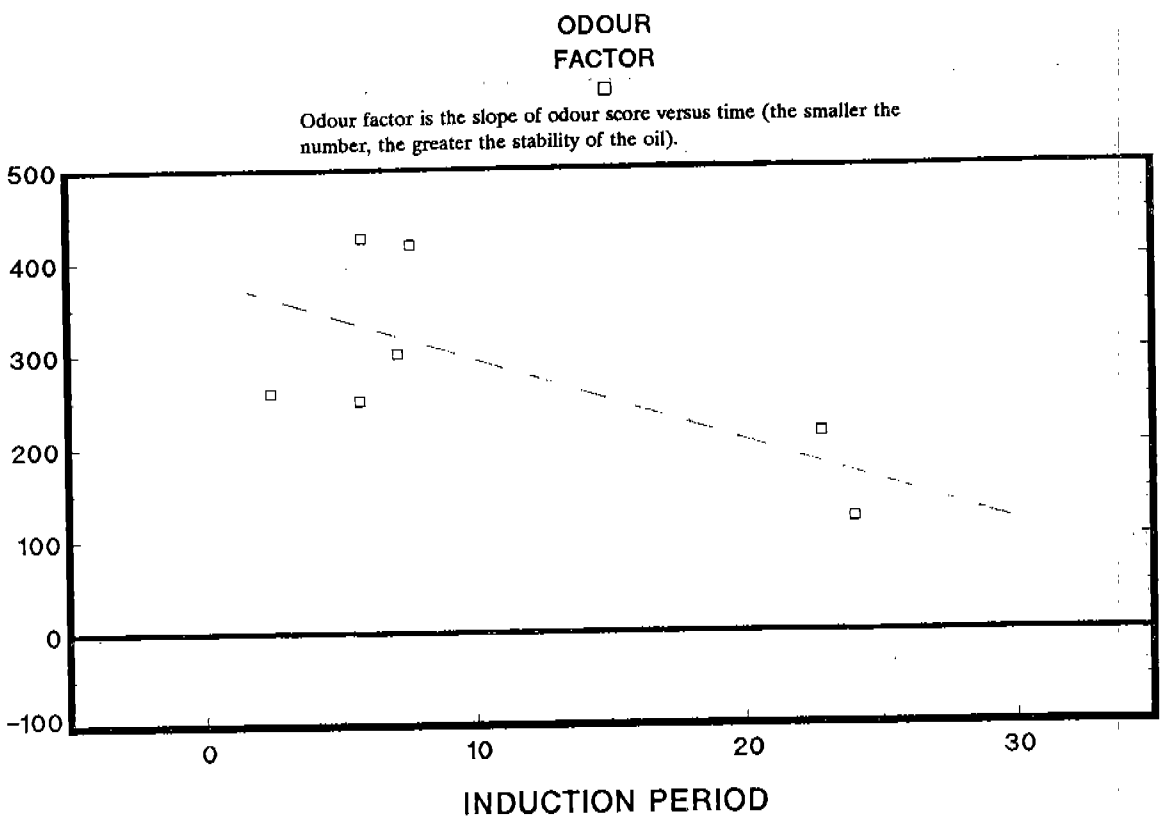

FIGURE 2

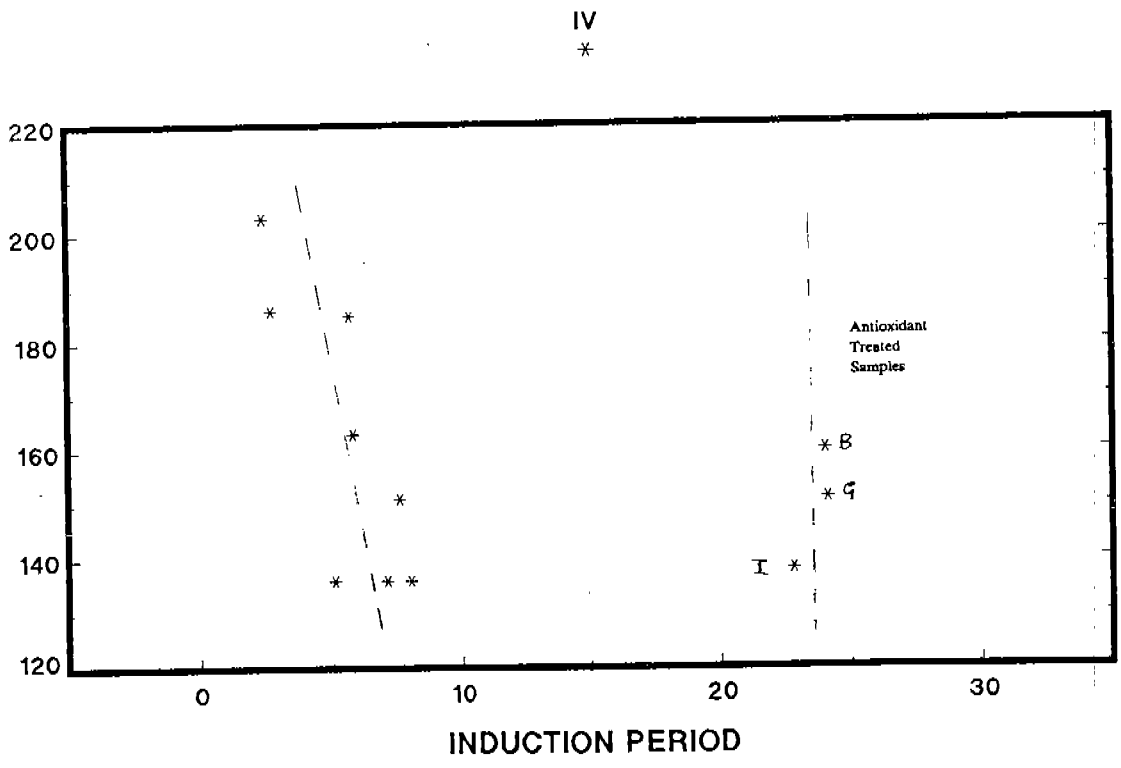

FIGURE 3

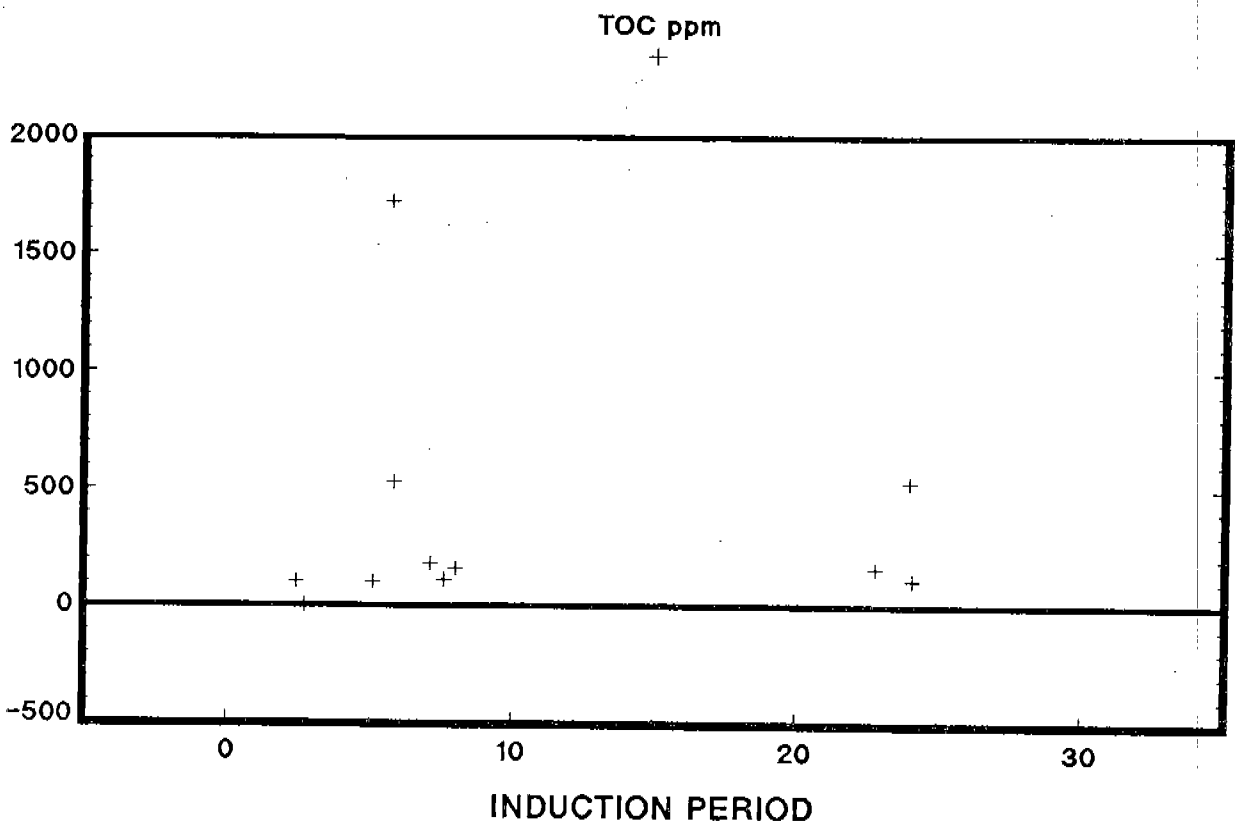

FIGURE 4

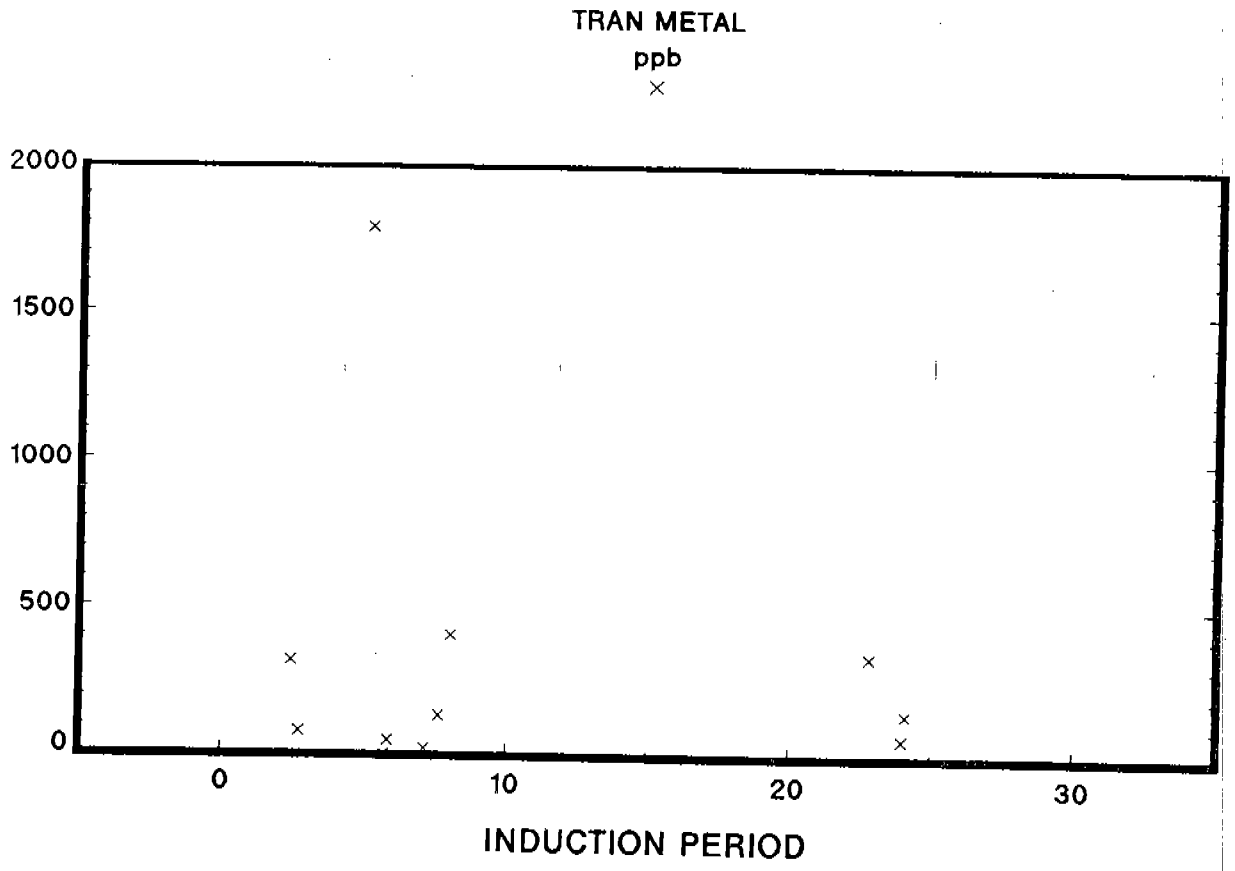

FIGURE 5

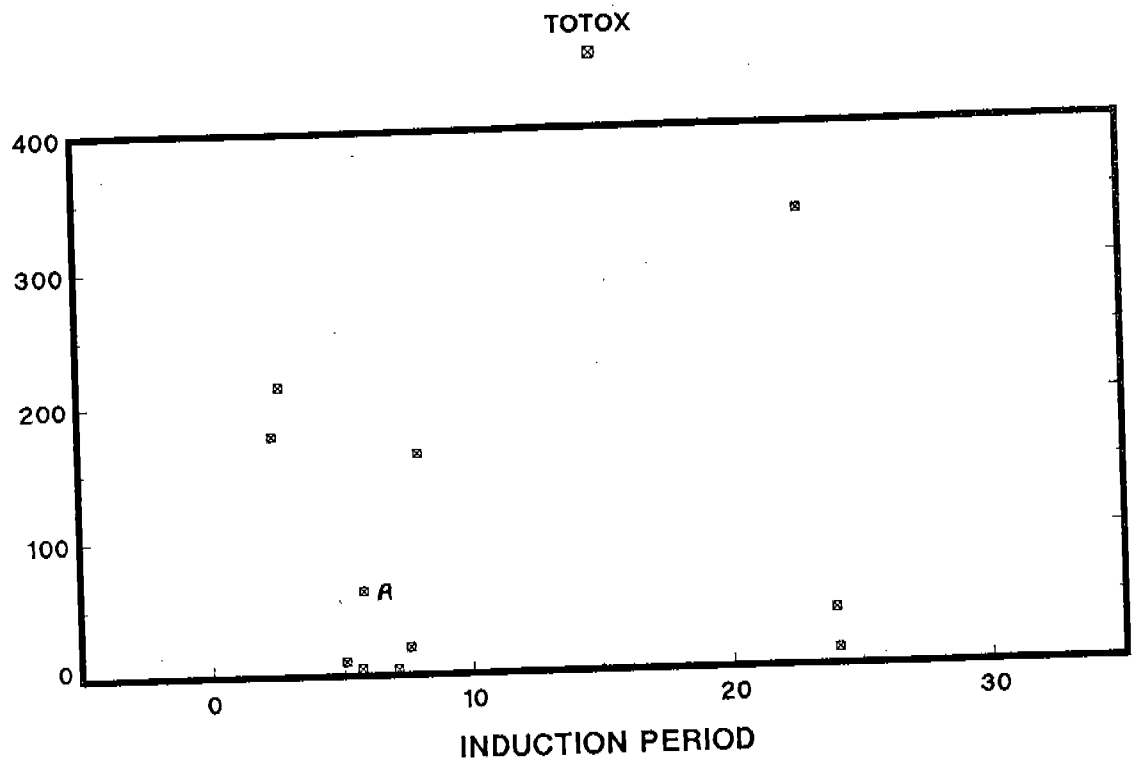

FIGURE 6

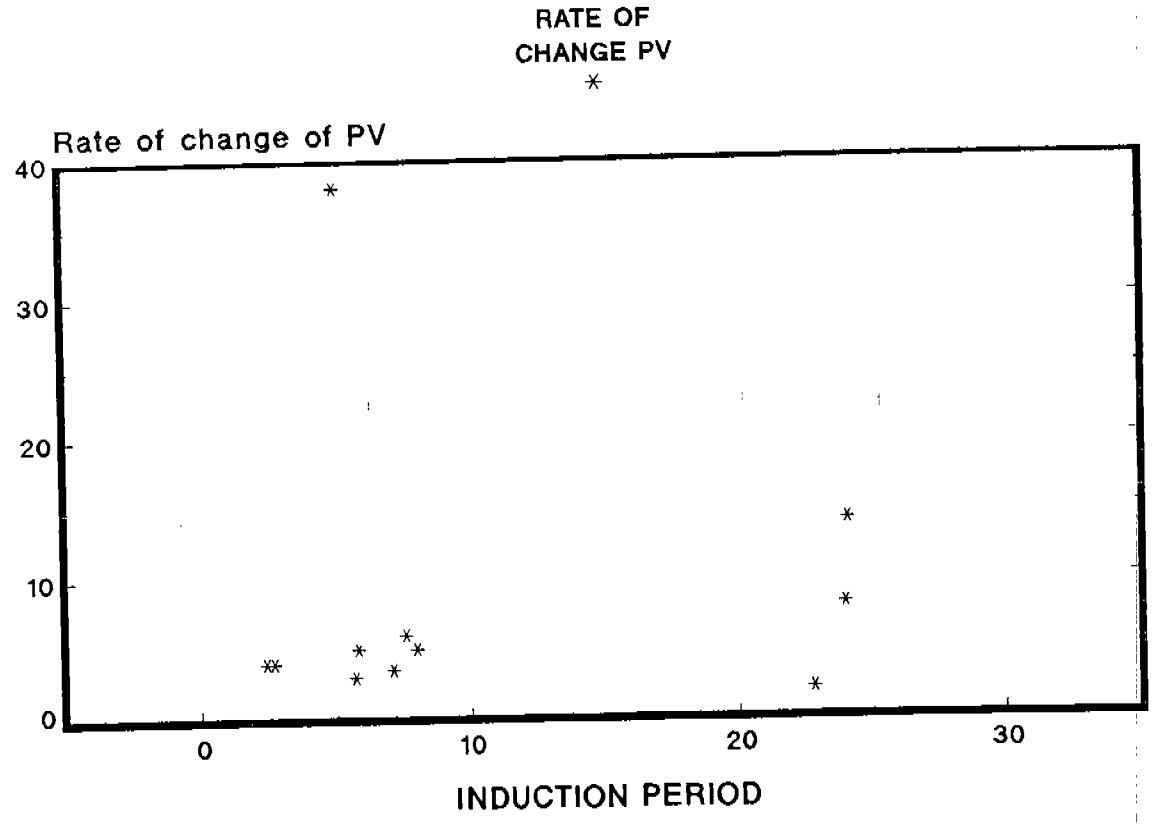

Table 1. Chemical analysis of fish oil "A".

Analysis	Values
Iodine Value (wijs)	163.0
Free Fatty Acids, % as 22:6	
Initial	0.2
Final	0.2
Fatty Acids, mg per 100 mg lipid	
14:0	7.9
16:0	21.0
16:1	10.5
18:0	1.0
18:1	13.1
18:2	1.3
18:3	1.2
18:4	3.9
20:1	1.5
20:5	13.9
22:1	0.8
22:4	0.6
22:5	2.2
22:6	13.7
Total	92.6
Iron	46.8 ppb
Copper	3.5 ppb
Tocopherols(∂)	530.0 ppm
Retinol	nd
Carotenoids	nd

nd-none detected

Table 2. Sensory and oxidative stability of fish oil "A" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number	Sensory[b] Odor	Taste	TBA Value
Initial	13.1	0.0	13.1	8.3	8.4	0.3
2 days	14.3	3.5	21.3	7.1	6.9	0.6
4 days	15.9	5.3	26.5	6.2	6.0	1.0
6 days	15.7	11.5	38.7	4.4	5.8	2.1
8 days	17.3	18.5	54.3	6.3	5.9	1.7
10 days	17.7	23.0	63.7	5.3	5.1	2.1
13 days	21.3	20.5	62.3	2.8	4.9	2.8

Induction period: 5.8 hr. (70 C).

[a]Totox number = 2 (peroxide value) + anisidine value.

[b]Sensory: odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor; taste scores based on 10-1 scale: 10 = bland, 1 = extreme intensity

Table 3. Chemical analysis of fish oil "B"

Analysis	Values
Iodine Value (wijs)	159.9
Iron	56.7 ppb
Copper	5.3 ppb

This is the same oil as "A", but with antioxidants.

Table 4. Sensory and oxidative stability of fish oil"B" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number	Sensory[b] Odor	Sensory[b] Taste	TBA Value
Initial	12.8	1.3	15.3	9.3	9.0	0.2
1 week	12.9	10.9	34.8	7.6	7.1	0.5
2 weeks	14.1	18.1	50.3	5.1	7.1	0.7
3 weeks	14.0	27.5	69.0	6.9	5.9	0.8
4 weeks	12.2	29.4	71.0	6.1	5.6	1.1
5 weeks	16.8	50.0	98.8	3.4	4.8	1.3
6 weeks	18.3	63.0	144.3	4.7	4.9	3.3

Induction period: 24.0 hr. (70 C).
Schaal oven test: 24.5 hr.

[a]Totox number = 2 (peroxide value) + anisidine value.

[b]Sensory: odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor; taste scores based on 10-1 scale: 10 = bland, 1 = extreme intensity.

Table 5. Chemical analysis of fish oil "C".

Analysis	Values
Iodine Value (wijs)	185.0
Free Fatty Acids, % as 22:6	
Initial	0.1
Final	0.1
Fatty Acids, mg per 100 mg lipid	
14:0	7.1
16:0	16.1
16:1	7.8
18:0	2.0
18:1	11.9
18:2	1.0
18:3	0.6
18:4	2.6
20:1	2.9
20:5	19.2
22:1	1.9
22:4	0.6
22:5	2.7
22:6	14.7
Total	91.1
Iron	4.5 ppb
Copper	nd
Tocopherols	
(α)	650 ppm
(β)	70 ppm
(δ)	750 ppm
(γ)	250 ppm
Retinol	nd
Carotenoids	nd

nd-none detected

Table 6. Sensory and oxidative stability of fish oil "C" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number	Sensory[b] Odor	Sensory[b] Taste	TBA Value
Initial	0.9	0.0	0.9	8.8	8.6	0.2
3 days	2.3	3.1	8.5	7.2	8.3	0.3
5 days	3.0	4.9	12.8	7.5	8.3	0.4
7 days	3.0	12.6	28.2	5.8	6.3	0.6
9 days	4.2	23.3	50.8	6.3	6.8	1.0
11 days	4.3	30.7	65.7	6.9	6.2	1.6
15 days	9.6	49.4	108.4	6.1	4.9	3.6

Induction period: 5.7 hr. (70 C).

[a]Totox number = 2 (peroxide value) + anisidine value.

[b]Sensory: Odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor; taste scores based on 10-1 scale: 10 = bland, 1 = extreme intensity.

Table 7. Chemical analysis of fish oil "D".

Analysis	Values
Iodine Value (wijs)	203.1
Free Fatty Acids, % as 22:6	
Initial	0.1
Final	0.1
Fatty Acids, mg per 100 mg lipid	
14:0	6.9
16:0	18.0
16:1	7.7
18:0	1.4
18:1	13.6
18:2	1.3
18:3	1.0
18:4	2.7
20:1	0.9
20:5	14.8
22:1	0.3
22:4	0.3
22:5	2.0
22:6	16.3
Total	87.2
Iron	270.6 ppb
Copper	44.0 ppb
Tocopherols(∂)	100 ppm
Retinol	nd
Carotenoids	nd

nd-none detected

Table 8. Sensory and oxidative stability of fish oil "D" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number	Sensory[b] Odor	Taste	TBA Value
Initial	21.6	2.5	26.6	5.8	6.4	0.9
2 days	22.8	6.2	35.2	6.6	5.9	1.3
3 days	24.0	8.2	40.4	4.7	5.5	1.1
4 days	24.5	11.5	47.5	5.4	5.7	1.6
6 days	26.4	18.4	63.2	6.2	5.8	2.1
8 days	27.3	23.3	73.9	5.5	6.1	2.3
10 days	30.7	28.1	86.9	4.8	5.4	1.4
13 days	30.0	30.7	91.4	4.0	4.4	1.2
16 days	34.5	50.7	135.9	3.1	4.4	2.7

Induction period: 2.4 hr. (70C).

[a]Totox number = 2 (peroxide value) + anisidine value.

[b]Sensory: odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor; taste scores based on 10-1 scale: 10 = bland, 1 = extreme intensity.

Table 9. Chemical analysis of fish oil "E".

Analysis	Values
Iodine Value (wijs)	135.5
Fatty Acids, mg/100 mg lipid	
14:0	5.9
16:0	13.0
16:1	7.6
18:0	0.6
18:1	14.0
18:2	1.5
18:3	0.7
18:4	5.8
20:1	13.5
20:5	7.3
22:1	20.1
22:4	0.3
22:5	0.4
22:6	5.8
Total	96.5
Iron	16.3 ppb
Copper	8.6 ppb
Tocopherols(∂)	180.0 ppm
Retinol	nd
Carotenoids	nd

nd-none detected

Table 10. Sensory and oxidative stability of fish oil "E" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number	Sensory[b] Odor	Sensory[b] Taste	TBA Value	E_{232}	E_{270}
Initial	1.4	0.0	1.4	9.5	9.8	0.1	–	–
2 days	1.9	1.6	5.1	9.4	9.6	0.2	4.6	–
4 days	2.3	3.1	8.5	9.0	8.6	0.2	6.7	–
7 days	3.4	9.5	22.4	7.5	7.8	0.4	6.9	1.6
11 days	4.8	22.0	48.8	6.2	5.8	0.7	7.8	1.8
13 days	6.4	30.0	66.4	4.4	4.5	1.4	7.9	2.0
16 days	8.3	35.6	79.5	4.2	3.9	2.1	8.7	1.9
19 days	9.6	42.9	95.4	3.2	3.7	2.1	9.7	2.0

Induction period: 7.1 hr. (70 C).

[a]Totox number = 2 (peroxide value) + anisidine value.

[b]Sensory: odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor; taste scores based on 10-1 scale: 10 = bland, 1 = extreme intensity.

Table 11. Chemical analysis of fish oil "F"

Analysis	Values	
Iodine Value (wijs)	151.2	
Fatty Acids, mg per 100 mg lipid		
14:0	8.1	
16:0	15.0	
16:1	5.5	
18:0	nd	
18:1	9.9	
18:2	2.3	
18:3	1.7	
18:4	4.0	
20:1	9.5	
20:5	7.3	
22:1	15.4	
22:4	nd	
22:5	nd	
22:6	11.3	
Total	90.0	
Iron	133.3	ppb
Copper	0.4	ppb
Tocopherols(∂)	110	ppm
Retinol	nd	
Carotenoids	nd	

nd-none detected

Table 12. Sensory and oxidative stability of fish oil "F" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number	Sensory[b] Odor	Sensory[b] Taste	TBA Value	E_{232}	E_{270}
Initial	5.4	0.1	5.6	7.5	5.8	0.3	10.0	2.4
2 days	5.9	2.5	10.9	6.9	5.2	0.3	10.0	2.3
3 days	6.5	3.7	13.9	5.4	4.5	0.3	9.0	2.3
6 days	7.5	11.4	30.3	5.5	4.7	0.6	10.4	2.3

Induction period: 7.6 hr. (70 C).

[a]Totox number = 2 (peroxide value) + anisidine value.

[b]Sensory: odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor; taste scores based on 10-1 scale: 10 = bland, 1 = extreme intensity.

Table 13. Chemical analysis of fish oil "G".

Analysis	Values
Iodine Value (wijs)	150.5
Free Fatty Acids, % as 22:6	
Initial	0.1
Final	0.1
Iron	145.6 ppb
Copper	0.5 ppb

This oil is the same as oil "F", but with antioxidants.

Table 14. Sensory and oxidative stability of fish oil "G" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number	Sensory[b] Odor	Taste
Initial	5.5	0.6	8.1	6.3	5.0
9 days	5.8	6.1	18.0	7.2	—
15 days	7.3	10.9	29.1	6.6	—
21 days	7.5	13.4	34.3	—	—
29 days	8.2	22.7	53.5	—	—

Induction period: 24.1 hr. (70 C).

[a]Totox number = 2 (peroxide value) + anisidine value.

[b]Sensory: odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor; taste scores based on 10-1 scale: 10 = bland, 1 = extreme intensity.

Table 15. Chemical analysis of fish oil "H".

Analysis	Values
Iodine Value (wijs)	135.5
Free Fatty Acids, % as 22:6	
Initial	0.2
Final	0.2
Fatty Acids, mg per % mg lipid	
14:0	6.6
16:0	11.6
16:1	10.9
18:0	nd
18:1	13.8
18:2	1.2
18:3	nd
18:4	2.4
20:1	13.8
20:5	7.7
22:1	17.3
22:4	nd
22:5	nd
22:6	5.0
Total	90.3
Iron	384.9 ppb
Copper	19.5 ppb
Tocopherols(∂)	160.0 ppm
Retinol	0.5 ppm
Carotenoids	nd

nd-none detected

Table 16. Sensory and oxidative stability of fish oil "H" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number
Initial	16.6	8.0	32.6
2 days	18.2	12.7	43.6
5 days	18.2	20.3	58.8
7 days	18.8	22.1	63.0

Induction period: 8.0 hr. (70 C).
Initial Sensory: Odor : 4.4; taste: 4.3.
Odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor; taste scores based on 10-1 scale: 10 = bland, 1 = extreme intensity.
[a]Totox number = 2 (peroxide value) + anisidine value.

Table 17. Chemical analysis of fish oil "I".

Analysis	Values
Iodine Value (wijs)	137.5
Iron	300.8 ppb
Copper	34.4 ppb

This oil is the same as "H", but with antioxidants.

Table 18. Sensory and oxidative stability of fish oil "I" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number	Sensory[b] Odor
Initial	11.9	16.5	44.9	6.0
5 days	23.5	17.4	58.3	6.6
13 days	21.4	60.3	142.0	4.3
16 days	23.7	57.3	138.3	3.4

Induction period: 22.8 hr. (70 C).

[a]Totox number = 2 (peroxide value) + anisidine value.

[b]Sensory: odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor.

Table 19. Chemical analysis of fish oil "J".

Analysis	Values
Iodine Value (wijs)	136.3
Free Fatty Acids, % as 22:6 Initial	5.0
Fatty Acids, mg/ 100 mg lipid	
14:0	7.5
16:0	14.8
16:1	8.9
18:0	0.98
18:1	12.8
18:2	1.8
18:3	nd
18:4	8.6
20:1	18.5
20:5	7.7
22:1	5.2
22:4	0.22
22:5	nd
22:6	8.1
Total	95.1
Iron	1779.0 ppb
Copper	7.3 ppb
Tocopherols(∂)	100.0 ppm
Retinol	1.4 ppm
Carotenoids*	45.7 ppm

nd-none detected

*Concentration estimated using an $E^{1\%}_{1cm}$ at 466 nm=2200 (canthaxanthin).

Table 20. Sensory and oxidative stability of fish oil "J" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number
Initial	9.2	1.8	12.8
4 days	9.1	5.3	19.7
8 days	9.7	6.8	23.3
13 days	9.7	6.4	22.5
22 days	8.8	9.2	27.2
32 days	10.4	10.9	32.2
43 days	11.8	20.9	53.6

Induction period: 5.1 hr. (70C).

[a]Totox number = 2 (peroxide value) + anisidine value.

Initial sensory score (odor): 1.2. Odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor.

Table 21. Chemical analysis of fish oil "K".

Analysis	Values
Iodine Value (wijs)	186.8
Free Fatty Acids, % as 22:6	
Initial	0.3
Final	0.3
Fatty Acids, mg per 100 mg lipid	
14:0	8.6
16:0	18.9
16:1	11.8
18:0	2.9
18:1	9.8
18:2	0.99
18:3	0.59
18:4	2.7
20:1	2.3
20:5	18.1
22:1	0.1
22:4	nd
22:5	nd
22:6	13.7
Total	90.6
Iron	75.4 ppb
Copper	2.4 ppb
Tocopherols	nd
Carotenoids	nd

nd-none detected

Table 22. Sensory and oxidative stability of fish oil "K" stored at room temperature.

Storage Time	Anisidine Value	Peroxide Value	TOTOX[a] Number
Initial	24.3	5.7	35.7
2 days	26.1	10.7	47.5
4 days	27.7	16.8	61.3
8 days	29.6	25.7	81.0
11 days	31.4	36.1	103.6

Induction period: 2.7 hr. (70 C).

[a]Totox number = 2 (peroxide value) + anisidine value.

Initial sensory score (odor): 1.3. Odor scores based on 10-1 scale: 10 = no odor, 1 = strong odor.

Table 23. Phosphorus content of fish oils.

Fish Oils	Phosphorus content (mg/kg)*	% phospholipid in oil†
A	2.74	0.007
C	3.30	0.008
D	2.45	0.006
E	9.58	0.023
G	3.92	0.009
H	3.14	0.007
J	12.80	0.031
K	12.70	0.031

*Phosphorus was determined by hydrolysis with 2M HCl at 120C (A.J. de Koning and Theodora Mol. 1989. A Convenient Method For the Accurate Determination of Phosphorus in Fish Oils. Fat Sci. Technol. 91(1). 36)

†calculated assuming a molecular weight of the phospholipid of 750

Table 24. Induction periods of fish oils at 70C.

Code	Induction period, hr.
A	5.8
B	24.0
C	5.7
D	2.4
E	7.1
F	7.6
G	24.1
H	8.0
I	22.8
J	5.1
K	2.7

Oxidation was measured by anisidine value.