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Changes in the quality of fish oils due to storage temperature and time

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Abstract

The effects of storage temperature on oil quality of various fishes were examined. Fish oils extracted from horse mackerel, shad, garfish and golden mullet were used in oil analyses. Crude fish oils obtained by a solvent extraction method were stored at +4 °C and −18 °C. Chemical quality of the oils was evaluated with various parameters, including iodine, ester, acid, saponification, peroxide, and thio-barbituric acid values and unsaponifiable matter at various time intervals for 150 days of storage. All quality parameters, except iodine and ester values, increased during storage at both of temperatures. Sample oils stored at +4 °C preserved acceptable characteristics for 90 days. Acceptability tolerance was found to be 120 days for shad oil and 150 days for golden mullet, garfish and horse mackerel oils stored at −18 °C. The highest oxidative deterioration was observed in shad oils. Among all samples, garfish oil showed the greatest stability against oxidation.

Keywords: Fish oil; Oil quality; Oxidation

1. Introduction

Fish oil has been around for a very long time (Bimbo, 1998). Recent studies have clearly shown the importance of omega −3 and −6 fatty acids for human health and nutrition (Connor, 2000; Kinsella, 1987; URL, 2002). Fortunately, fish oil is one of the best sources of dietary supply of these fatty acids. Oils and fats used for human consumption are natural components of vegetables and land or marine origin animals (Rafflenbeul, 2001). Regardless of sources, principal components of these oils and fats are the same. They are triglyceride oils in that their principal constituents, amounting, usually to over 90% of their total composition, are the tri-esters of fatty acids and glycerol. The remainder of their composition consists of partial glycerides, such as mono- and di-glycerides, free fatty acids (FFA) and other compounds, in small quantities. The differences between the various oils and fats lie in the fatty acid composition of the triglycerides and phosphatides, and in the composition of the unsaponifiable fraction and other minor compounds (Young, 1986a). Fish species contain oil in different amounts, varying according to species, age, gender, location, species-origin characteristics, such as spawning and migration seasons, and also some environmental conditions, such as temperature (Borgstrom, 1961; Huss, 1988).

Recently, fish oil became popular because of its useful effects on human health. Many studies have shown that fish oil has important roles in prevention of cardiovascular diseases and some types of cancer, including colon, breast and prostate (Marchioli, 2001, 2002; URL, 2002). In addition, fish oil also helps to prevent brain aging and Alzheimer's disease (Kyle, 1999). Many health experts suggest that two to three servings per week of seafood should be consumed in order to meet the recommended level of essential fatty acids for pregnant wo-

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men, children and elderly people (Huhges, 1995; Olsen & Secher, 2002). Seafood is the best source of dietary supply of omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It has been suggested that consuming EPA and DHA may reduce the risk of mortality from cardiovascular disease in people who have already experienced a cardiac event (URL, 2002).

The main purpose of this study was to investigate the stability of fish oil during storage. Fish oil has important industrial applications in food, pharmacy, cosmetics and paint products. Because of these wide areas of applications, quality and stability of fish oil have gained more importance. Fish oil spoils in two major ways, like oils from animal and vegetable sources: oxidative spoilage and hydrolytic spoilage (Cmolik & Pokorny, 2000). Due to its high content of polyunsaturated fatty acids, including EPA and DHA, fish oil is highly susceptible to oxidative spoilage (Huss, 1988) and the rate of fish oil oxidation is significantly different from that of other oils. Fish oils also include high concentrations of phospholipids, containing unsaturated fatty acids, which make them even more sensitive than other oils. The break in the induction curve is less sharp, and the beginning of the increase in the peroxide value occurs sooner (Liston, Stansby, & Olcott, 1963; Stansby, 1967). The course of oxidation is often quite different between extracted fish oils and lipids in fish tissues (Liston et al., 1963). The autoxidation of fish oils is the most important cause of deterioration in quality (Lundberg, 1967). Undesirable flavours and odours develop at very low peroxide values at an early stage of oxidation, even during the induction period (Stansby, 1967, 1982).

For evaluation of oil stability and monitoring of deterioration during storage, commonly used methods include peroxide value (PV), thiobarbituric acid (TBA), acid value (AV) and unsaponifiable matter (USM). There are some important limits posed by regulatory agencies for quality and acceptability of oils for human consumption; for example, 8 meq O_2/kg of oil is the limit of acceptability of PV and, for TBA value, this limit is 7–8 mg malonaldehyde/kg of oil (Huss, 1988). The focus of the study was to investigate deterioration tolerance of fish oils extracted from various fish species commonly consumed in Turkey which include horse mackerel, shad, garfish, and golden mullet. These species have great commercial importance, constituting approximately 20% of the total fishing in Turkey (Anonymous, 2001).

2. Materials and methods

2.1. Fish species and chemicals

The fish species used for this study were horse mackerel (*Trachurus trachurus*, L., 1758), shad (*Alosa fallax,* Lacepede, 1803), garfish (*Belone belone*, L., 1758) and golden mullet (*Mugil auratus*, Risso, 1810), which were obtained from a local marketplace (about 4–5 kg fish for each species) in December 2002, in a sexually matured state. All chemicals used were of analytical grade and obtained from Merck (Germany), Sigma–Aldrich (USA) and J.T. Baker (USA).

2.2. Sample preparation

Upon arrival of the fish at the marketplace, they were transported to the laboratory, washed with cold water, eviscerated and filleted immediately. All species were minced and homogenized separately and dried at 103 ± 2 °C overnight until reaching constant weight. Homogenized and dried fishes were stored at 0 °C prior to the oil extraction. In order to obtain sufficient fish oil, all species were continually used for extraction until the desired amount was collected. The total time spent for the extraction was less than four days. Extraction duration, allowed for each sample, was no more than 2 h, to speed up the process. For this purpose, a Soxhlet system was used for semi-continuous extraction, and *n*-hexane was used as solvent. For each species, about 300 ml oil were collected. When the extraction of all species had finished, oil samples were divided into two groups and contained in dark-coloured bottles for two different storage temperatures (2 bottles for each oil sample and of total of 8 bottles). The first group was stored in a refrigerator at +4 °C while the other group was stored in a deep freezer at −18 °C. In order to carry out oil analyses during storage for 150 days, aliquots were taken from each bottle at various time intervals and used for chemical analysis.

2.3. Chemical analysis

Ester value (EV) and acid value (AV) were determined according to procedures in Official and Standardized Methods of Analysis (Watson, 1994). Iodine value (IV), peroxide value (PV) and saponification value (SV) were done by titration while unsaponifiable matter (USM) was determined gravimetrically according to the AOCS method (AOCS, 1992). TBA was analyzed by a distillation method (Tarladgis, Watts, & Younathen, 1960). All analyses were done in duplicate.

2.4. Statistical analysis

All data were evaluated with MINITAB statistical software using ANOVA and Tukey's test to compare the data for each oil sample for significant change (95% confidence interval) (Sokal & Rolf, 1974). In all tables, statistical significance is indicated by appropriate letters within the tables.

3. Results and discussion

Fish oil, containing high level of polyunsaturated fatty acids, is very labile to hydrolytic spoilage and especially oxidative deterioration at a varying velocity, strongly depending on storage conditions and fatty acid profile. In the present study, deterioration rates of oils, of various fishes frequently consumed in Turkey, were determined under different storage conditions. The fish species used for oil extraction were garfish, golden mullet, shad and horse mackerel, which have quite considerable commercial importance in Turkey and other neighbouring countries, including Georgia and Ukraine. Commonly employed chemical analyses, such as PV, TBA, EV, AV, IV, SV and USM, were carried out to monitor oil quality under defined storage conditions.

The results of all chemical analyses for garfish, golden mullet, shad and horse mackerel are presented in Tables 1–4, respectively. In general, it was observed, in all oil samples, that PV, TBA and AV gradually increased, while IV and EV decreased during incubation at both temperatures. Decreasing IV indicates a reduction of oil unsaturation, while decrease in EV shows an increase in FFA due to hydrolysis of oils. The observed decreases in IV and EV were considerably lower at −18 °C than at 4 °C during storage.

There were gradual decreases in IV for all oil samples, except for horse mackerel oil data on the 60th day compared to that of the 30th day stored at +4 °C that may be an analytical variation. Generally, IV of all samples were decreased, that is unsaturation degrees of samples were decreased with time at both storage temperatures. However, this occurred in lower amount at −18 °C. Storage temperatures showed statistical significance ($P < 0.05$) on IV variation of horse mackerel and garfish oil and no statistical significance for the other two oil samples.

SVs of samples were increased at both storage temperatures; the biggest increase occurred in garfish stored at +4 °C (Table 1). SV is a measure of molecular weight and defined as the amount of alkali required to saponify fatty acids in a given weight of oil. Hydrolysis and oxidation bring about lipid breakdown, forming free fatty acids or aldehydes and ketones as the end-products. During this process an increase in SV, is not normally expected. However, it is possible that the end-products of oxidation, such as aldehydes and ketones, may contribute to increase in SV.

EV has no acceptability limit for human consumption, but gives valuable information for interpretting triglyceride degradation. During the course of storage, EVs of oil samples decreased at both temperatures, like IV. EVs for all oil samples showed comparable decreases but the greatest change was in golden mullet oil stored at +4 °C (Table 2), which is also reflected in AV change in golden mullet oil compared with other oil samples stored at +4 °C.

USMs for all oil samples were increased from ≈0.1 to 4.5 during storage at +4 °C. The changes were smaller at −18 °C. The unsaponifiable fraction of fish oil is composed primarily of sterols, glyceryl ethers, hydrocarbons and fatty alcohols, including minor quantities of pigments, vitamins and various oxidation products. However, there seems to be a difference in the meaning and interpretation of USM among fish oils. Young (1986b) reported that the unsaponifiable content of fish oils was normally less than 2% of fish oil, and the value would increase to 8% of fish oil under certain seasonal and feeding conditions. However, according to Bimbo (1998), there is no standard for USM of fish oil and this

Table 1
Changes in the quality of garfish oil stored at two different temperatures

Days of Storage	IV (mg I/g oil)	PV (meq O_2/kg oil)	TBA (mg MA/kg)	AV (mg KOH/g)	EV (mg KOH/g)	SV (mg KOH/g)	USM (%)
at +4 °C							
1	177^{a}	3.65^{a}	1.20^{a}	3.25^{a}	177^{a}	182^{a}	0.75^{a}
15	175^{a}	3.90^{b}	3.40^{b}	4.90^{b}	174^{a}	185^{ab}	1.25^{b}
30	166^{b}	4.90^{c}	4.20^{c}	6.08^{c}	169^{b}	187^{b}	2.15^{c}
60	166^{b}	6.25^{d}	5.40^{d}	7.83^{d}	163^{c}	188^{b}	3.15^{d}
90	161^{c}	8.10^{e}	6.20^{e}	8.65^{e}	157^{d}	193^{c}	3.80^{ef}
120	160^{c}	9.80^{f}	9.30^{f}	10.47^{f}	153^{d}	194^{c}	4.20^{fg}
150	162^{c}	10.60^{g}	9.70^{f}	14.09^{g}	154^{d}	195^{c}	4.50^{g}
at −18 °C							
1	177^{a}	3.65^{a}	1.20^{a}	3.25^{a}	177^{a}	182^{ab}	0.75^{a}
15	175^{ab}	3.90^{b}	3.20^{b}	4.03^{b}	175^{a}	183^{ab}	1.10^{b}
30	171^{c}	4.20^{bc}	3.90^{c}	4.58^{c}	173^{b}	181^{a}	1.25^{bc}
60	173^{bc}	4.55^{c}	4.15^{c}	5.08^{d}	171^{bc}	185^{bc}	1.55^{cd}
90	170^{c}	4.30^{bc}	4.40^{c}	5.81^{e}	169^{cd}	185^{bc}	1.90^{de}
120	172^{bc}	5.10^{d}	5.20^{d}	7.61^{f}	167^{de}	188^{c}	2.30^{ef}
150	171^{c}	5.90^{e}	5.90^{d}	9.93^{g}	165^{e}	190^{c}	2.40^{f}

Same letter in each column determines no statistical difference separately between values of oils stored at the two temperatures.

Table 2
Changes in the quality of golden mullet oil stored at two different temperatures

Days of Storage	IV (mg I/g oil)	PV (meq O_2/kg oil)	TBA (mg MA/kg)	AV (mg KOH/g)	EV (mg KOH/g)	SV (mg KOH/g)	USM (%)
at +4 °C							
1	184[a]	3.15[a]	1.00[a]	1.74[a]	182[a]	176[a]	0.85[a]
15	181[a]	3.70[b]	3.15[b]	3.83[b]	180[a]	178[ab]	1.15[ab]
30	183[a]	4.20[c]	3.35[b]	4.95[c]	172[b]	178[ab]	1.35[b]
60	174[b]	5.60[d]	5.15[c]	6.10[d]	170[b]	182[c]	2.85[c]
90	169[c]	7.20[e]	6.30[d]	8.02[e]	159[c]	184[cd]	3.80[d]
120	170[bc]	9.30[f]	8.80[e]	10.1[f]	155[cd]	185[de]	4.10[de]
150	167[c]	11.3[g]	9.40[e]	13.2[g]	153[d]	187[e]	4.40[e]
at −18 °C							
1	184[a]	3.15[a]	1.00[a]	1.74[a]	182[a]	176[a]	0.85[a]
15	181[ab]	3.50[ab]	2.35[b]	2.64[b]	181[a]	176[a]	0.85[a]
30	179[bc]	3.70[b]	2.85[c]	3.27[c]	178[b]	178[ab]	1.10[ab]
60	182[b]	4.15[d]	3.25[cd]	4.15[d]	173[c]	177[a]	1.25[b]
90	177[cd]	4.40[d]	3.80[d]	5.03[e]	171[cd]	178[ab]	1.90[c]
120	175[d]	5.30[e]	5.70[e]	6.97[f]	168[d]	181[bc]	2.40[d]
150	176[cd]	6.10[f]	6.20[e]	9.54[g]	164[e]	182[c]	2.60[d]

Same letter in each column determines no statistical difference separately between values of oils stored at the two temperatures.

Table 3
Changes in the quality of shad oil stored at two different temperatures

Days of Storage	IV (mg I/g oil)	PV (meq O_2/kg oil)	TBA (mg MA/kg)	AV (mg KOH/g)	EV (mg KOH/g)	SV (mg KOH/g)	USM (%)
at +4 °C							
1	197[a]	6.30[a]	1.90[a]	4.63[a]	181[a]	191[a]	1.25[a]
15	195[b]	6.80[ab]	4.15[b]	5.35[b]	177[b]	195[b]	1.70[b]
30	188[c]	7.10[b]	4.75[c]	6.15[c]	175[b]	197[bc]	2.10[c]
60	189[d]	8.65[c]	6.30[d]	8.07[d]	168[c]	198[bc]	3.40[d]
90	183[e]	9.40[d]	6.70[e]	9.28[e]	160[d]	198[bc]	4.10[e]
120	178[f]	10.8[e]	8.70[f]	10.4[f]	161[d]	199[c]	4.50[f]
150	176[g]	11.9[f]	11.3[g]	13.4[g]	159[d]	200[c]	4.70[f]
at −18 °C							
1	197[a]	6.30[a]	1.90[a]	4.63[a]	181[a]	191[a]	1.25[a]
15	196[b]	6.70[b]	2.65[b]	4.86[b]	181[a]	192[a]	1.55[ab]
30	193[c]	6.90[bc]	4.25[c]	5.27[c]	180[a]	191[a]	1.55[ab]
60	194[d]	7.20[cd]	5.15[d]	5.82[d]	173[b]	194[ab]	1.85[bc]
90	190[e]	7.10[bd]	4.80[d]	5.94[d]	170[bc]	194[abc]	2.10[cd]
120	188[f]	7.60[d]	6.10[e]	8.19[e]	168[c]	197[bc]	2.30[de]
150	187[g]	8.10[e]	8.70[f]	9.61[f]	167[c]	198[c]	2.70[e]

Same letter in each column determines no statistical difference separately between values of oils stored at the two temperatures.

parameter varies greatly depending on fish species. It has been suggested that the upper limit for USM should be 3.5% for top quality, beyond that the price of fish oil is reduced (Anonymous, 1986). As seen in the tables, USM of all oil samples stored at +4 °C exceeded or reached the acceptability limit for human consumption in 90 days. On the other hand, oil samples stored at −18 °C did not exceed the acceptability limit for 150 days. Based on our results, we recommend an acceptability limit for USM of crude fish oil to be 4% of fish oil for human nutritional purposes. The recommendation represents the deterioration amount indicated by other chemical quality parameters.

PV, AV and TBA values of all oil samples showed similar patterns, comparable to one another. It has been reported that PV of sardine oil stored at −20 °C increased from 4.12 to 18.6 during frozen storage for 150 days (Verma, Srikar, Sudhakara, & Sarma, 1995). In another study, PV of whole fish of whiting (*M. mediterraneus*) and horse mackerel (*T. trachurus*) stored at −18 °C for 360 days showed an increase from 3.46 and 8.46 to 28.3 and 24.8, respectively (Simeonidou, Govaris, & Vareltzis, 1997). Karacam and Boran (1996) have reported that PVs of whole and gutted anchovy, stored at −18 °C for 180 days, increased from 1.8 to 8.2. In the present study, shad oil showed the greatest oxidative stability among all fish species (Table 3). Starting approximately from 30 days, the effect of storage temperature was statistically significant in all oil samples, measured as PV, AV and TBA (significances are not

Table 4
Changes in the quality of horse mackerel oil stored at two different temperatures

Days of Storage	IV (mg I/g oil)	PV (meq O_2/kg oil)	TBA (mg MA/kg)	AV (mg KOH/g)	EV (mg KOH/g)	SV (mg KOH/g)	USM (%)
at +4 °C							
1	189[a]	4.80[a]	2.15[a]	3.97[a]	174[a]	193[a]	1.00[a]
15	185[b]	5.40[b]	2.70[b]	5.08[b]	171[ab]	195[b]	1.25[a]
30	181[c]	5.80[b]	4.10[c]	5.96[c]	163[c]	198[c]	1.75[b]
60	184[b]	7.15[c]	6.75[d]	7.24[d]	168[bd]	199[cd]	3.15[c]
90	176[d]	8.40[d]	6.90[d]	9.07[e]	165[cd]	201[de]	3.80[d]
120	177[d]	10.3[e]	10.2[e]	10.7[f]	166[cd]	201[e]	4.20[d]
150	169[e]	11.2[f]	11.8[f]	12.8[g]	163[c]	202[e]	4.30[d]
at −18 °C							
1	189[a]	4.80[a]	2.15[a]	3.97[a]	174[a]	193[a]	1.00[a]
15	189[a]	5.20[b]	3.10[b]	4.22[b]	172[abc]	192[a]	1.10[a]
30	187[ab]	5.10[ab]	2.45[c]	4.60[c]	170[bc]	194[ab]	1.25[a]
60	186[bc]	5.45[b]	3.70[d]	4.93[d]	172[abc]	193[ab]	2.10[b]
90	185[b]	6.10[c]	3.90[d]	5.68[e]	173[ab]	195[ab]	2.70[c]
120	183[bc]	6.20[c]	6.40[e]	7.03[f]	170[abc]	196[b]	3.20[cd]
150	182[b]	6.90[d]	7.60[f]	10.0[g]	168[c]	197[b]	3.40[d]

Same letter in each column determines no statistical difference separately between values of oils stored at the two temperatures.

indicated in tables). Young (1986a) has reported that PV of crude fish oil was between 3 and 20. In this study, PVs of all examined fish oil samples did not exceed 20 meq O_2/kg oil. According to Huss (1988), the acceptability limit for PV of crude fish oil is 7–8 meq O_2/kg oil. All examined oil samples stored at +4 °C reached this limit in 90 days. Samples stored at −18 °C did not exceed the acceptability limit within 150 days.

Like the PV and TBA, AVs for all oil samples, showed a marked increase for both storage temperatures. The biggest increase in AV of oil samples was seen in golden mullet. Increase in AV is generally associated with lipase activity originating from microorganisms or biological tissue. The acceptable limit for AV is reported to be 7–8 mg KOH (Bimbo, 1998). Our results showed that this limit was exceeded beyond 60 days of storage at +4 °C. It is important to note that we have not sterilized the samples nor studied them under aseptic conditions. Thus, it is possible that some enzyme or microorganism contamination might occur during sample removal. De Koning (1999) observed that hydrolysis of fish was greatly reduced upon sterilization. It was found that golden mullet oil was the most sensitive to hydrolytic deterioration among the examined oil samples (Table 4).

TBA values for oil samples also increased during storage, like AV and PV. Karacam and Boran (1996) reported that TBA values of whole or gutted anchovy increased from 0.3 to 3.1 during storage for 180 days at −18 °C. In another study, TBA of whole fish of *M. mediterraneus* and *T. trachurus* increased from 0.026 to 0.192 and from 0.129 to 0.264, respectively, after storage for 360 days at −18 °C. Among fish oils stored at +4 °C, it was observed that the largest increase occurred in horse mackerel oil; however, at −18 °C shad oil showed the greatest increase.

4. Conclusions

The effects of storage temperature on oil quality of various fish species were examined. It was found that the storage temperature had important effects on storage stability of fish oil. Fish oil samples stored at −18 °C had almost twice longer shelf life than had samples stored at +4 °C. At the same time, it was also found that oxidative as well as hydrolytic stabilities vary greatly, depending on fish species. Among fish species examined, garfish and shad showed the greatest stability against oxidation and preserved their acceptability properties for at least 150 days at −18 °C.

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References

Anonymous. (1986). *The production of fish meal and oil*. FAO Fisheries Technical Paper-142, Rome, Italy: FAO Fisheries Department.

Anonymous (2001). *Fisheries statistics*. State Institute of Statistics, Ankara, Turkey: Prime Ministry Republic of Turkey.

AOCS (1992). *Official methods and recommended practices of the American oil chemists' society*. 4th ed., Champaign, American Oil Chemists' Society, IL: USA.

Bimbo, A.P. (1998). *Guidelines for Characterizing Food-Grade Fish Oils*. Inform vol. 9, No. 5, Hertfordshire: UK.

Borgstrom, G. (1961). *Fish as food, production, biochemistry and microbiology* (i). London, UK: Academic Press Inc..

Cmolik, J., & Pokorny, J. (2000). Physical refining of edible oils. *European Journal of Lipid Science and Technology, 102*, 472–486.

Connor, W. E. (2000). Importance of $n-3$ fatty acids in health and disease. *American Journal of Clinical Nutrition, 71*, 171S–175S.

De Koning, A. J. (1999). The free fatty acid content of fish oil, part V. The effect of microbial contamination on the increase in free fatty acid content of fish oils during storage at 25 °C. *Fett/Lipid, 101*(5), 184–186.

Huhges, D. A. (1995). Fish oil and the immune system. *Nutrition & Food Science, 95*(2), 12–16.

Huss, H. H. (1988). *Fresh fish quality and quality changes*. Rome, Italy: FAO.

Karacam, H., & Boran, M. (1996). Quality changes in frozen whole and gutted anchovies during storage at −18 °C. *International Journal of Food Science and Technology, 31*, 527–531.

Kinsella, J. E. (1987). *Sea foods and fish oils in human health and disease*. New York, USA: Marcel Dekker.

Kyle, D. J. (1999). Low serum docosahexaenoic acid is a significant risk factor for alzheimer's dementia. *Lipids, 34*, S245.

Liston, J., Stansby, M. E., & Olcott, H. S. (1963). *Bacteriological and chemical basis for deteriorative changes. Industrial fishery technology*. New York: Reinhold Publishing Corp., p. 260–261.

Lundberg, W. O. (1967). General deterioration reactions. In M. E. Stansby (Ed.), *Fish oils: Their chemistry, technology, stability, nutritional properties and uses* (pp. 141–147). Westport, Conn: Avi Publishing Co..

Marchioli, R. (2001). Efficacy of *n*-3 polyunsaturated fatty acids after myocardial infarction: Results of gissi-prevenzione Trial. *Lipids, 36*, S119–S126.

Marchioli, R. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: Time-course analysis of the results of GISSI-prevenzione. *Circulation, 105*(23), 1897–1903.

Olsen, S. F., & Secher, N. J. (2002). Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: Prospective cohort study. *British Medical Journal, 324*(23), 1–5.

Rafflenbeul, W. (2001). Fish for a healthy heart. *European Journal of Lipid Science and Technology, 103*, 315–317.

Sokal, R.R., Rolf, F.J. (1974). *Introduction to Biostatistics*. W.H. FREEMAN (Ed), (2nd ed.), New York, USA.

Simeonidou, S., Govaris, A., & Vareltzis, K. (1997). Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (Trachurus trachurus) and Mediterranean Hake (Merluccius mediterraneus). *Z Lebensm Unters Forsch A, 204*, 405–410.

Stansby, M. E. (1967). Odors and flavors. In M. E. Stansby (Ed.), *Fish Oils: Their chemistry, technology, stability, nutritional properties and uses* (pp. 171–180). Westport, Conn: Avi Publishing Co..

Stansby, M. E. (1982). Properties of fish oils and their application to handling of fish and to nutritional and industrial use. In R. E. Martin, G. J. Flick, C. E. Hebard, & D. R. Ward (Eds.), *Chemistry and biochemistry of marine food products* (pp. 75–92). Westport, Conn: Avi Publishing Co..

Tarladgis, B. G., Watts, B. M., & Younathen, M. T. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of American Oil Chemists' Society, 37*, 44–48.

URL (2002). http://www.oilofpisces.com/fishnews.html Fish Oil and Angina/Heart Attack, Health Benefits of Fish Oils.

Verma, J. K., Srikar, L. N., Sudhakara, N. S., & Sarma, J. (1995). Effects of frozen storage on lipid freshness parameters and some functional properties of oil sardine (*Sardinella longiceps*) Mince. *Food Research International, 28*(1), 87–90.

Watson, C. A. (1994). *Official and standardized methods of analysis* (Third Ed.). Cambridge, UK: The Royal Society of Chemistry.

Young, F.V.K. (1986a). *The refining and hydrogenation of fish oil*. Fish Oil Bulletin, Vol. 17, International Association of Fish Meal Manufacturers, Hertfordshire,UK: St. Alban's.

Young, F.V.K. (1986b). *The chemical and physical properties of crude fish oils for refiners and hydrogenators*. Fish Oil Bulletin, Vol. 18, Liverpool, UK.