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MiniReview
Importance and production of omega-3 fatty acids from natural sources

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Abstract: Omega-3 fatty acids are essential polyunsaturated fatty acids that must be obtained through food. The review includes the details of Omega-3 PUFA their importance to humans. Omega-3 PUFA concentrates provide a useful alternative for the intake of required amount of fatty acids. The production techniques for Omega-3 concentrates have been extensively discussed with their advantages and application. Also an insight has been provided for improvement of the shortcomings of these production techniques. With growing public awareness of the health benefits of Omega-3 fatty acids, this market is expected to grow in near future. This overview provides a discussion of the various health benefits, production techniques and their application for commercial production. The review also discusses plant sources as a good source of Omega−3 fatty acids which must be exploited.

Keywords: Omega-3, fatty acids, natural sources

Introduction

Omega-3 fatty acids (*n*−3 fatty acids or ω−3 fatty acids) are a family of unsaturated fatty acids that have in common a final carbon-carbon double bond at the *n*-3 position that is, the third bind from methyl end of the fatty acid. The human body cannot synthesize *n*−3 fatty acids de novo but it can form 20-carbon unsaturated *n*−3 fatty acids (like EPA) and 22-carbon unsaturated *n*−3 fatty acids (like DHA) from the eighteen-carbon *n*−3 fatty acid α-linolenic acid. These conversions occur competitively with *n*−6 fatty acids, which are essential closely related chemical analogues that are derived from linoleic acid. Both the *n*−3 α-linolenic acid and *n*−6 linoleic acid are essential nutrients which must be obtained from food. Synthesis of the longer *n*−3 fatty acids from linolenic acid within the body is competitively slowed by the *n*−6 analogues. Thus accumulation of long-chain *n*−3 fatty acids in tissues is more effective when they are obtained directly from food or when competing amounts of *n*−6 analogs do not greatly exceed the amounts of *n*−3. It is advised that pregnant women and mothers, nursing mothers, young children and women who might become pregnant require *n*-3 fatty acids but cant eat several types of fish. Thus, a natural source of *n*-3 fatty acids must be explored and included in quality dieatry supplements.

Chemistry of omega-3 fatty acids

Among the various omega-3 fatty acids (Table 1) there are three essential omega-3 fatty acids important nutritionally to humans include α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). α-linolenic acid (18:3, *n*−3; ALA), eicosapentaenoic acid (20:5, *n*−3; EPA), and docosahexaenoic acid (22:6, *n*−3; DHA) (Figure 1).

Table 1. List of most common *n*−3 fatty acids found in nature

Common name	Lipid name	Chemical name
n/a	16:3 (*n*−3)	*all-cis*-7,10,13-hexadecatrienoic acid
α -Linolenic aicd (ALA)	18:3 (*n*−3)	*all-cis*-9,12,15-octadecatrienoic acid
Stearidonic acid (SDA)	18:4 (*n*−3)	*all-cis*-6,9,12,15-octadecatetraenoic acid
Eicosatrienoic acid (ETE)	20:3 (*n*−3)	*all-cis*-11,14,17-eicosatrienoic acid
Eicosatetraenoic acid (ETA)	20:4 (*n*−3)	*all-cis*-8,11,14,17-eicosatetraenoic acid
Eicosapentaenoic acid (EPA)	20:5 (*n*−3)	*all-cis*-5,8,11,14,17-eicosapentaenoic acid
Docosapentaenoic acid (DPA), Clupanodonic acid	22:5 (*n*−3)	*all-cis*-7,10,13,16,19-docosapentaenoic acid
Docosahexaenoic acid (DHA)	22:6 (*n*−3)	*all-cis*-4,7,10,13,16,19-docosahexaenoic acid
Tetracosapentaenoic acid	24:5 (*n*−3)	*all-cis*-9,12,15,18,21-docosahexaenoic acid
Tetracosahexaenoic acid (Nisinic acid)	24:6 (*n*−3)	*all-cis*-6,9,12,15,18,21-tetracosenoic acid

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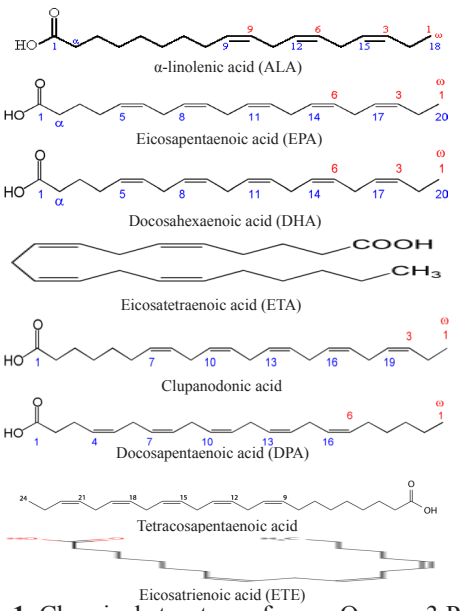

Figure1. Chemical structure of some Omega-3 PUFA found in nature

These three polyunsaturates have 3, 5 or 6 double bonds in a carbon chain of 18, 20 or 22 carbon atoms, respectively. All double bonds are in the *cis* -configuration; i.e the two hydrogen atoms are on the same side of the double bond. Most naturally-produced fatty acids (created or transformed in plant cells with an even number of carbon in chains) are in *cis*-configuration where they are more easily transformable. The *trans*-configuration results in much more stable chains those are very difficult to further break or transform, forming longer chains that aggregate in tissues and lack the necessary hydrophilic properties. Moreover the *n*−3 compounds are still more fragile than *n*−6 because the last double bond is geometrically and electrically more exposed, notably in the natural *cis*-configuration.

The omega-3 (or *n*-3) and omega-6 (or *n*-6) families based on the position of the first double bond from the methyl end group in the fatty acid chain. This is because the position of the double bond from the methyl end influences the biological activity of these molecules involved. Therefore, LA and ALA are regarded as the parent *n*-6 and *n*-3 fatty acids in each series, respectively.

Metabolism of n-3 and n-6 fatty acids

The enzymes responsible for desaturation and chain elongation in both *n*-3 and *n*-6 families are identical (Figure 2). Imbalance in the intake of *n*-6 versus *n*-3 fatty acids sometimes leads to over-production of eicosanoids with less preferred activities. Eicosanoids made from *n*−3 fats are often referred to as anti-inflammatory, but in fact they are just less pro-inflammatory than those made from *n*−6 fats. If both *n*−3 and *n*−6 are present, they will "compete" to be transformed, so the ratio of *n*−3:*n*−6 directly affects the type of eicosanoids that are produced. This led to greater interest in finding ways to control the synthesis of *n*−6 eicosanoids. The simplest way would be by consuming more *n*−3 and fewer *n*−6 fatty acids. F Guebre-Egziabher and his team studied the effect of diet modification to decrease the *n*−6/*n*−3 PUFA ratio on cardiovascular risk factors and resting energy expenditure. Their studies showed that a decreased *n*−6/*n*−3 PUFA ratio can be achieved with simple dietary counseling, resulting in multiple, potentially favourable effects on the metabolic and inflammatory profiles. In 1982 Dr. Charles Serhan's group at Harvard discovered that the EPA is responsible for the formation of potent antiinflamatory nanomolecules, called Resolvins in the human body. Later his team discovered that omega-3 fatty acids are converted into other antiinflammatory molecules called Maresins and Omega-3-oxylipins, which partly explain the versatile health effects of *n*-3 enriched foods.

It has been reported that conversion of ALA to EPA and further to DHA in humans is limited, but varies with individuals. Generally, women have higher ALA conversion efficiency than men, probably due to the lower rate of utilization of dietary ALA for beta-oxidation. This suggests that biological engineering of ALA conversion efficiency is possible. However, Goyens *et al*. argue that it is the absolute amount of ALA, rather than the ratio of *n*−3 and *n*−6 fatty acids, which affects the conversion. (Goyens *et al*., 2006).

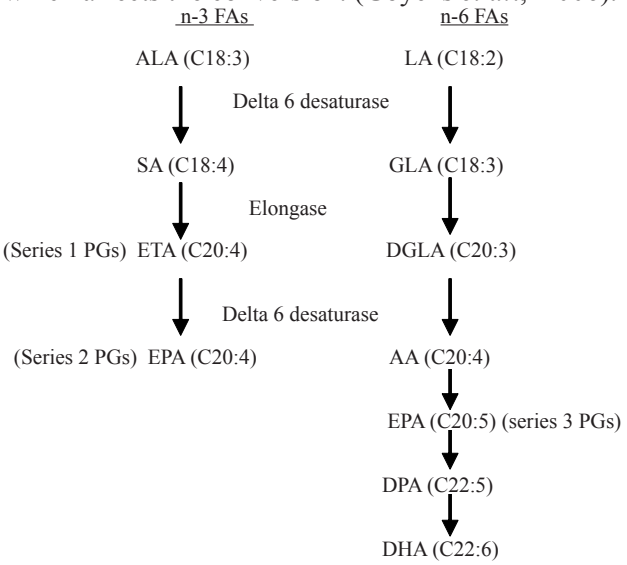

Figure 2. Essential fatty acids metabolism

Production technologies of omega-3 fatty acids concentrate

As an alternative to the commonly used dietary foods (table 2) as a source of omega-3 fatty acids, supplementation has been recommended. For clinical applications, generally capsules containing fish oils,

especially fish liver oils have been used. The dosage required to carry out the desired pharmacological effect carries the risk of vitamin A and D overdose and subsequent toxic effects as well as increase in the intake of cholesterol and other fatty acids. Moreover, there are ethical concerns regarding use and killing of fish. Therefore, concentrated forms of ω-3 PUFA from natural sources must be developed (Haagsma *et al*., 1980). Further advantage of concentrated forms of ω-3 PUFA is that they are devoid of saturated and monosaturated fatty acids. This keeps the daily intake of lipids as low as possible.

Hence, commercial production of ω-3 PUFA concentrates with high percentages of EPA and DHA from natural source is a major challenge. There are several techniques for production of ω-3 PUFA but only few can be used for cost effective and large scale production to meet the growing demand.

Table 2. List of some common foods as a source of omega 3 fatty acids

Food	Rating	Botanical source	Family
Flaxseeds	excellent	*Linum Usitatissimum*	Linaceae
Cloves, dried, ground	very good	*Eugenia caryophyllata*	Myrtaceae
Walnuts	excellent	*Juglans nigra*	Juglandaceae
Cauliflower, boiled	very good	*Brassica, oleracea*	Brassicaceae
Mustard seeds	very good	*Brassica Juncea*	Brassicaceae
Broccoli, steamed	very good	*Brassica oleraceae* L.	Cruciferae
Spinach, boiled	good	*Spinacia oleracea*	Amaranthaceae
Soybeans, cooked	good	*Glycine max*	Fabaceae
Turnip greens, cooked	good	*Brassica rapa*	Brassicaceae
Strawberries	good	*Fragaria ananassa*	Rosaceae
Raspberries	good	*Rubus idaeus* L.	Rosaceae

Chromatographic methods

It is possible to separate fatty acids according to their number of carbon and their degree of unsaturation by using appropriate adsorbents (Brown *et al*., 1955). High performance liquid chromatography (Beebe *et al*., 1988) and silver resin chromatography (Belarbi hassan *et al*., 1999) have been used for production of ω-3 PUFA concentrates. Solvent choice for seperation of fatty acid esters depends on the desired purity of eluted fractions and their use as well as production requirements (Tokiwa *et al*., 1981). Higher purity fractions of EPA and DHA were obtained using tetrahydrofuran (THF) (Krzynowek *et al*., 1988). However THF is undesirable because it's potentially explosive and its peroxides initiate oxidative decomposition of PUFA. Ethanol and water would be the solvents of choice if the end product is to be consumed by humans. Solvent choice for the separation of the fatty esters depends on the desired purity of the eluted fractions and their use as well as production requirements.

Adolf *et al*. (1985), fractionated methyl esters containing 29.1% EPA and 20.5% DHA into fractions of 87.7% into fractions of 87.7% EPA and 95.4% DHA with increasing amounts of acetonitrile (0-30%) in methanol. The separation was done isocratically using 40% acetonitrile in acetone; one of the fractions contained approximately 69% of total EPA and DHA after being concentrated.

Distillation method

Distillation has been used for partial separation of mixtures of fatty acids esters. This method takes advantage of the differences in the boiling point and molecular weight of fatty acids under reduced pressure (Brown *et al*., 1995). This technique requires high temperatures of approximately 250°C (Berger *et al*., 1979). Short-path distillation or molecular distillation uses lower temperatures and shot heating intervals. The most widely used distillation technique is fractional distillation under reduced pressure (0.1-1mm HG). Even under these conditions moderately high temperatures are required sufficient to cause oxidation, polymerization and isomerization of double bonds of ω-3 PUFA. Commonly used heated columns packed with glass helices or metal packing have a disadvantage of significant hold up and pressure drop through the column. Spinning band column may be used to overcome these disadvantages. Privett *et al*., found marked decomposition of arachidonic acid when it was distilled slowly in a spinning band column. Hence, design of a method for preparation of ω-3 PUFA concentrates which involves low process temperature and time to minimize thermal damage is desirable.

Low temperature crystallization

Low-temperature crystallization was originally developed to separate certain fatty acids, TAG, esters and other lipids which are highly soluble in organic solvents at temperatures above 0°C, but become sparingly soluble at temperatures down to minus 80°C (Brown *et al*., 1995). The solubility of fats in organic solvents decreases with increasing mean molecular weight and increases with increasing unsaturation (Chawala *et al*., 1990). At low temperature long chain fatty saturated fatty acids which have higher melting point crystallize out and PUFA remain in the liquid form.

Low-temperature crystallization process may be carried out in the absence of a solvent or in a selected solvent/solvent mixture. The commonly used solvents are methanol and acetone which have been employed to separate stearic and oleic fractions. Crystallization in the absence of solvent occurs using

the Tritiaux process, which involves slow cooling and slow agitation in the hydrophillization process. In this process, crystallization produces slurry of solid and liquid components, the latter being enriched with Omega-3 fatty acids. It has been reported that use of different organic solvents affects the concentration of PUFA (Yokochi *et al*., 1990). Therefore, proper choice of solvent is necessary to achieve the optimum concentration yield of ω-3 PUFA.

Han *et al*. (1987), have found that alkali salts of less unsaturated fatty acids crystallize more rapidly than those of PUFA containing four or more double bonds, when saponified solution is cooled. They also compared the cooling temperature and the rate of cooling on enrichment of ω-3 PUFA. Fatty acid composition of the prepared concentrates indicated that cooling rate and temperature had little influence on the yield and contents of EPA and DHA. Therefore, use of ambient temperatures would offer a practical choice for large-scale separation and production of ω-3 PUFA concentrates.

Enzymatic methods

Enzymes involved are lipases which catalyse estrification, hydrolysis or exchange of fatty acids in esters (Marangoi *et al*., 1995). The direction and the choice of the reaction can be influenced by the choice of experimental conditions (Yadwad *et al*., 1991). The reaction is reversible and under low water activity conditions, the enzyme functions 'in reverse' that is synthesis of an ester bond rather than its hydrolysis (Miller *et al*., 1988).

Lipase-catalysed hydrolysis

The presence of *cis*-carbon-carbon double bonds in fatty acids results in bending of the chains. Therefore, the terminal methyl group of the fatty acids lies close to the ester bond which may cause a steric hindrance effect on lipases. The high bending effect of EPA and DHA due to presence of the 5 and 6 double bonds respectively enhances the steric hindrance effect. Therefore, lipases cannot reach the ester-linkage between these fatty acids. However, saturated and monounsaturated fatty acids do not present any such barriers to lipases and get easily hydrolysed. Thus, fatty acid selectivity of a lipase for EPA and DHA allows separation and concentration of these fatty acids from others in the remaining portion (Bottino *et al*., 1967). In addition, lipases have been frequently used to discriminate between EPA and DHA in concentrates containing both of these fatty acids, thus providing the possibility of producing ω-3 PUFA concentrates with dominance of either EPA or DHA.

Lipase-catalysed esterification

The TAG from PUFA is considered to be nutritionally more favourable than methyl or ethyl esters of fatty acids because experimental results have shown impaired intestinal absorption in laboratory animals (Harnazaki *et al*., 1982). In the modern world scenario triacylglycerols of PUFA are often promoted as being more natural than other fatty acid derivatives.

Supercritical fluid extraction

Supercritical fluid extraction (SPE) is relatively new seperation process that may circumvalent some of the problems accosiated with the use of convectional seperation techniques. A large number of gases are known to posses desirable solvent properties when raised to pressure (1000 to 2000 psig) above their critical values. The region in which a substance exists as a supercritical fluid is defined by its critical pressure (P_c) and critical temperature (T_c). Supercritical fluids offer an attractive choice for extraction and fractionation of a variety of raw materials. For food commodities, CO_2 is chosen because it has moderate critical temperature and pressure (31.1ºC, 1070 psig) and is inert, inexpensive, non-inflammable, environmentally acceptable, readily available and safe. The separation of PUFA by SPE is dependent on the molecular size of the components involved rather than their degree of unsaturation, therefore a prior concentration step is needed to achive a high concentration of PUFA in the final product (Mishra *et al*., 1993). Moreover, use of extremely high pressures and high capital costs might limit the widespread use of this method for concentrate production to larger companies. Recently propane has gained more interest in extraction technology especially in the nutraceutical industry (Pinton 1998). However, more research and development will be required to determine the extent and use for seperation of ω-3 PUFA from natural sources.

Urea complexation

Urea alone crystallizes in a tightly packed tetragonal structures with channels of 5.67 Å in diameter. However, in the presence of long straight chain molecules it crystallizes in a hexagonal structure with channels of 8-12 Å in diameter (Smith *et al*., 1952). The channels formed in the presence of long straight chain molecules are sufficiently large to accommodate aliphatic chains (Figure 3).

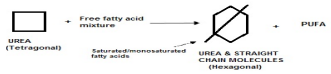

Figure 3. Formation of urea crystals in urea complexation technique

Monoenes are more readily complexed as compared to dienes which in turn, are more readily complexed than trienes. Hence, the stability of fatty acids- urea adducts parallels the geometry of the molecules involved. Formation of urea inclusion compounds depends on the degree of unsaturation of the fatty acids. Many publications describe the application of urea complexation as an analytical as well as preparative tool in fatty acid chemistry. Moreover, this method is also favoured because complexation depends upon the configuration of the fatty acid due to presence of multiple bonds rather than pure physical properties like melting point or solubility. The free fatty acids are mixed with an alcoholic solution of urea and then allowed to cool to a particular temperature depending on the degree of concentration desired. The saturated fatty acids are crystallized with urea (UCF) and the non-crystallized fatty acids (NUCF) in the solution can be separated by filtration. The liquid or the NUCF is enriched with ω-3 PUFA.

Han *et al*. (1987) have tested a series of solvents like ethanol, methanol, water and acetonitrile) as wetting agents for urea. Water has been the solvent of choice because of its low cost and lack of toxicity.

Pharmacology

Through an inefficient enzymatic process of desaturation (the rate of conversion is less than 1 percent), ALA produces EPA (20 carbons) and DHA (22 carbons), precursors to a group of eicosanoids that are anti-inflammatory, anti-thrombotic, anti-arrhythmic and vasodilator. The longer chain fatty acid derivative of linoleic acid is arachidonic acid (20 carbons), which is a precursor to a different group of eicosanoids that are proinflammatory and prothrombic. ALA as well as linoleic acid use and compete for the same enzymes in the production of their longer chain fatty acids, EPA, and arachidonic acid.

Uses and efficacy

Sudden death

Sudden death caused by sustained ventricular arrhythmias accounts to 50-60 percent of all deaths in persons with coronary heart disease (CHD) (Leaf *et al*., 2003).

Hyperlipidemia

Omega-3 fatty acids lower plasma triglyceride levels, particularly in persons with hypertriglyceridemia (Harris *et al*., 1997), by inhibiting the synthesis of very-low-density lipoprotein (VLDL) cholesterol and triglycerides in the liver.

Hypertension

Omega-3 fatty acids appear to have a dose response hypotensive effect in patients with hypertension and have little to no effect in normotensive patients (Howe *et al*., 1997).

Depression

An emerging area of research is examining the neurobehavioral aspects of omega-3 fatty acids (alpha-linolenic, eicosapentaenoic, docosahexaenoic) and the critical role of these essential fats in the functioning of the central nervous system. Investigations have linked omega-3 fatty acids to a number of neuropsychiatric disorders, including depression (Silvers *et al*., 2002).Further research is necessary before firm conclusions can be drawn regarding the neurobiological influences of omega-3 fatty acids and their clinical value in the treatment of depression. It is anticipated that additional research will shed further light on the neuropsychological aspects of dietary lipids.

Rheumatoid arthritis

Several small studies have found that ω-3 PUFA significantly reduced morning stiffness and the number of tender, swollen joints in patients with rheumatoid arthritis. It has been reported that reducing dietary (Cleland *et al*., 1998) intake of omega-6 fatty acids while increasing consumption of omega-3 fatty acids reduces the anti-inflammatory mediators of rheumatoid arthritis and, consequently, allows some patients to reduce or discontinue use of non steroidal anti-inflammatory drugs (Volker *et al*., 200).

Interactions and adverse effects

Omega-3 fatty acids exert a dose-related effect on bleeding time; however, there are no documented cases of abnormal bleeding as a result of fish oil supplementation, even at high dosages and in combination with other anticoagulant medications (Eritsland *et al*., 1996). Other potential side effects of omega-3 fatty acids include a fishy after taste and gastrointestinal disturbances, all of which appear to be dose-dependent (Kris-Etherton *et al*., 2002).

Dosage

The FDA has concluded that dietary dosages of up to 3 g per day of omega-3 fatty acids from marine sources are "Generally Recognized as Safe." For persons who are vegetarians or non-fish eaters, a total daily intake of 1.5 to 3 g per day of ALA seems to be beneficial (Kris-Etherton *et al*., 2002).

Conclusion

Omega-3 fatty acids have been part of human diet throughout evolution. Production of ω-3 PUFA may be achieved using a number of techniques. These products may be in the form of free fatty acids, methyl and ethyl esters or acylglycerols. Production by urea complexation is more common as the final product is in the form free acid or simple ester. Due to potential benefits of having the final product in the form acylglycerol, enzymatic procedures have gained momentum in industrial production. The ω-3 PUFA are generally obtained from fish oils. The plant sources provide a good alternative so that ω-3 PUFA concentrates can be developed as supplements. This also eliminates the ethical concerns regarding killing of fish.

References

Adolf, R.O. and Emiken, E. A. 1985. The isolation of omega 3 fatty acids and methyl esters of fish oils by silver resin chromatography Journal of the American Oil Chemists' Society 62:1592-1595.

Albert, C. M., Hennekens, C. H., O'Donnell, C. J., Ajani, U. A., Carey, V. J., Willett, W. C. 1998. Fish consumption and risk of sudden cardiac death. Journal Of the American Medical Association 279:23-28.

Appel, L. J., Miller, E. R., Seidler, A. J. and Whelton, P. K. 1993. Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical trials. Archives of Internal Medicine 153:1429-1438.

Bang, H. O. and Dyerberg, J. 1972. Plasma lipids and lipoproteins in Greenlandic West-coast Eskimos. Acta Medical Scandinavia l92:85-94.

Bang, H. O., Dyerberg, J. and Hjorne, N. 1976.The composition of food consumed by Greenland Eskimos. Acta Medical Scandinavia 200:69-73.

Beebe, L. M., Brown, P. R. and Turcotte, L. G. 1988. Preparative scale high performance liquid chromatography of omega 3 fatty acids esters derived from fish oils. Journal of Chromatography 495:369-378.

Berger, R. and Mcpherson, W. 1979. Fractional Distillation Journal of the American Oil Chemists' Society 56:743A-746A.

Bottino, N. R., Vandenberg, G. A. and Reiser, R. 1967. Resistance of certain long-chain polyunsaturated Fatty acids of marine oils to pancreatic lipase Hydrolysis. Lipids 2:489-493.

Brown, L. B. and Klob, D. X. 1955. Application of low temperature crystallization in separation of fatty acids and their compounds.Progress in Lipid Research 3:57-94.

Burr, M. L., Fehily, A. M., Gilbert, J. F., Rogers, S., Holliday, R. M. and Sweetnam, P. M. 1989. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 2:757-761.

Chawala, P. and DeMan, J. M. 1990. Measurement of size distribution of fat crystals using a laser particle counter. Journal of the American Oil Chemists' Society 76,329-332.

Cleland, L. G., French, J. K., Betts, W. H., Murphy, G. A. and Elliott, M. J. 1988. Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. Journal of Rheumatology 15:1471-1475.

Dyerberg, J., Bang, H. O. and Stofferson, E. l978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. Lancet 2:117-119.

Eritsland, J., Arnesen, H., Gronseth, K., Fjeld, N. B. and Abdelnoor, M. 1996. Effect of dietary supplementation with n-3 fatty acids on coronary artery bypass graft patency. American Journal of Cardiology 77:31-36.

Guallar, E., Aro, A., Jimenez, F. J., Martin-Moreno, J. M., Salminen, I. and van't Veer, P. 1999. Omega-3 fatty acids in adipose tissue and risk of myocardial infarction: the EURAMIC study. Arteriosclerosis, Thrombosis, and Vascular Biology 19:1111-1118.

Haagsma, N., Gent, C. M., Luten, L. B., Jong, R. M. and Doorn, E. 1980. Preparation of omega 3 fatty acids concentrate from cod liver oil. Journal of the American Oil Chemists' Society 59:117-118.

Han, D. S., Ahn, H. B. and Shin, H. K. 1987. Seperation of EPA and DHA from fish oils by solubility Differences of fatty acids salts in ethanol. Korean Journal of Food Science and Technology 19:430-434.

Harnazaki, T., Hirai, A., Terano, T., Sajiki, J., Kondo, S., Fujita, T., Tamura, Y. and Kumagai, A. 1982. Effect of orally administered Ethyl Ester of eicosapentanoic acid on PGI like substance Production by rat aorta. Prostaglandins 23:557-567.

Harris, W. S., Ginsberg, H. N., Arunakul, N., Shachter, N. S., Windsor, S. L. and Adams, M. 1997. Safety and efficacy of Omacor in severe hypertriglyceridemia. Journal of Cardiovascular Risk 4:385-391.

Hixon, A. W. and Bockelmann, L. B. 1942. Liquid liquid extraction employing solvents in the region of their critical temperatures. American institute of chemical Engineers 38:891-930.

Hu, F. B., Bronner, L., Willett, W. C., Stampfer, M. J., Rexrode, K.M. and Albert, C. M. 2002. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. Journal Of the American Medical Association 287:1815-1821.

Howe, P. R. 1997. Dietary fats and hypertension. Focus on fish oil. Annals of the New York Academy of Sciences 8:339-352.

James, M. J. and Cleland, L. G. 1997. Dietary n-3 fatty acids and therapy for rheumatoid arthritis. Seminars in Arthritis and Rheumatism 27:85-97.

Keys, A., Anderson, T. and Grande, F. l957. Serum cholesterol response to dietary fat. Lancet 1:787 (letter).

Lau, C. S., Morley, K. D. and Belch, J. J. 1993. Effects

of fish oil supplementation on non-steroidal anti-inflammatory drug requirement in patients with mild rheumatoid arthritis— a double-blind placebo controlled study. Rheumatology 32:982-989.

Leaf, A. 1990. Cardiovascular effects offish oils. Beyond the platelet. Circulation 82:624-628.

Leaf, A., Kang, J. X., Xiao, Y. F. and Billman, G. E. 2003. Clinical prevention of sudden cardiac death by *n*-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by *n*-3 fish oils. Circulation 107:2646-2652.

Lees, R. S. and Karel, M. 1990. Omega-3 fatty acids in health and disease. Marcel Dekker. New York.

Marangoni, A. and Rousseau, D. 1995. Engineering triacylglycerol: the role of interestrification Trends in Food Science & Technology 6:329-335.

Miller, C., Austin, H., Posorske, L. and Gonzlez, J. 1988. characteristics of an immobilized lipase for omerial synthesis of esters. Journal of the American Oil Chemists' Society 65, 927-931.

Mishra, V. K., Temelli, F. and Ooraikul, B. 1993. Extraction and purification of omega 3 fatty acids with an emphisis on supercritical fluid extraction. Food Research International 26:217-227.

Morris, M. C., Sacks, F. and Rosner, B. 1993. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation 88:523-33.

Nordoy, A. and Goodnight, S. 1990. Dietary lipids and thrombosis. Arteriosclerosis; 10:149-63.

Norell, S. E., Ahlbom, A., Feychting, M. and Pedersen, N. L. l986. Fish consumption and mortality from coronary heart disease. British Medical Journal 293:426-429.

Phillipson, B. E., Rothrock, D. W., Connor, W. E., Harris, W. S. and Llingworth, D. R. 1985. Reduction of plasma Lipids, Lipoproteins and Apoproteins by dietary Fish Oils in patients with Hyperglyceridemia. New England Journal of Medicine 312:1210-1216.

Pinton, M. 1998. Propane and Super critical extraction. Practical short course on processing of nutraceuticals / functional foods. Chapter 16.

Silvers, K. M. and Scott, K. M. 2002. Fish consumption and self-reported physical and mental health status. Public Health Nutrition 5:427-431.

Sinclair, H. 1956. Deficiency of essential fatty acids and atherosclerosis, etcetera. Lancet 1:38l-383.

Smith, A. E. 1952. Crystal structure of the urea- hydrogen complexes. Acta Crystallographica 5:224-235.

Teshima, S., Khanazawa, A. and Tokiwa, S. 1978. Separation of PUFA by column chromatography by silver impregnated silica gel. Bulletin of the Japanese Society of Fisheries Oceanography 44:927.

Tokiwa, S., Kanazawa, A. and Teshima, S. 1981. Prepration of eicosapentanoic and docosahexanoic acids by reversed phase high performance liquid chromatography. Bulletin of the Japanese Society of Fisheries Oceanography 47:675-678.

Vargova, V., Vesely, R., Sasinka, M. and Torok, C. 1998. Will administration of omega-3 unsaturated fatty acids reduce the use of nonsteroidal antirheumatic agents in children with chronic juvenile arthritis? [Slovak] Casopis Lekaru Ceskych 137:651-3.

Wanasaundara, U. N. and Shahidi, F. 1997. lipase assisted concentration of omega 3 faty acids in acylglycerols from marine oils. Journal of the American Oil Chemists' Society 75:945-951.

Woodman, R. J., Mori, T. A., Burke, V., Puddey, I. B., Watts, G. F. and Beilin, L. J. 2002. Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension. American Journal of Clinical Nutrition 76:1007-1015.

Yadwad, V. B., Ward, O. P. and Noronha, L. C. 1991. Application of lipase to concentrate the docosahexanoic acid fraction of fish oil. Biotechnology and Bioengineering 38:956-959.