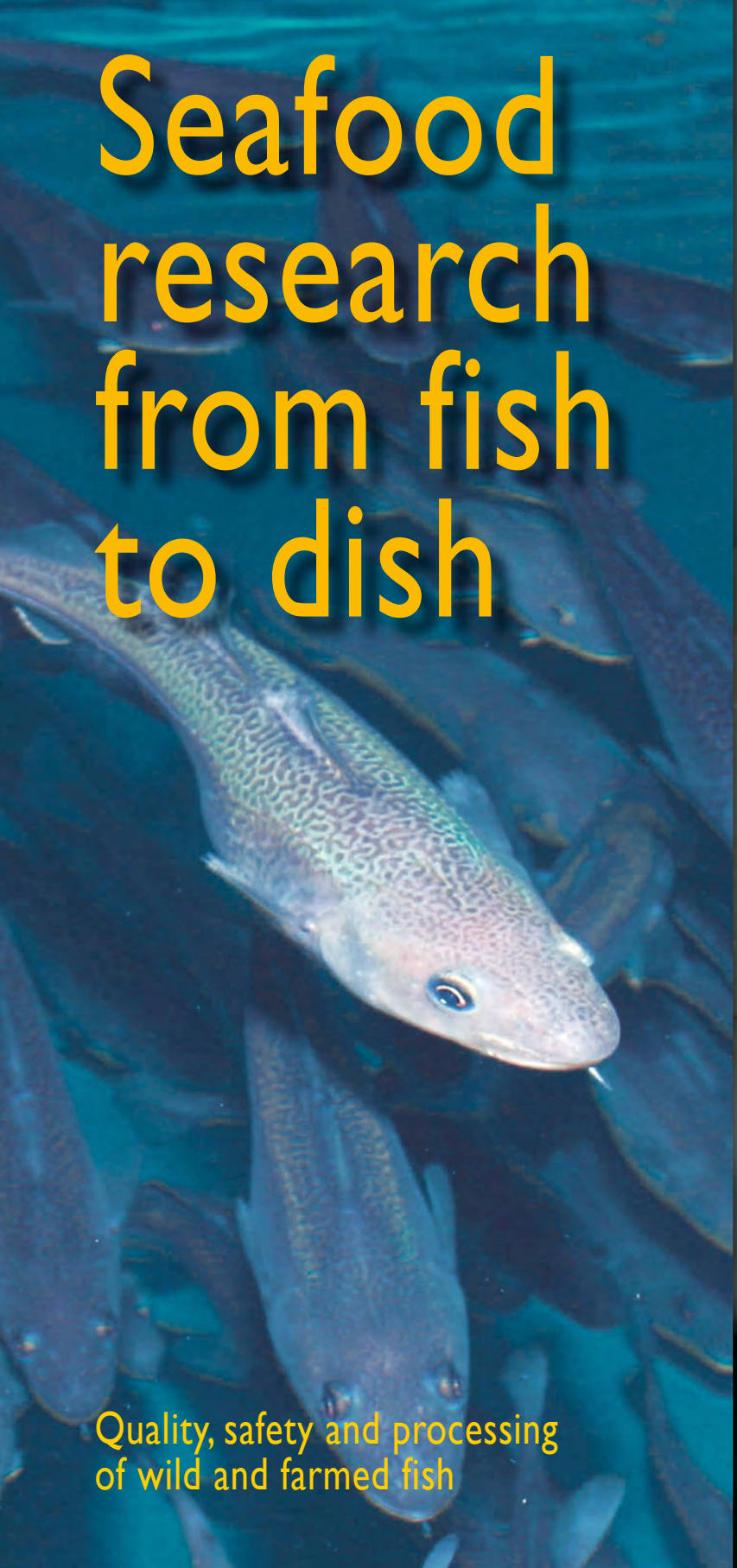
Seafood research from fish to dish



Quality, safety and processing of wild and farmed fish

edited by:
J.B. Luten
C. Jacobsen
K. Bekaert
A. Sæbø
J. Oehlenschläger

Seafood research from fish to dish

Seafood research from fish to dish

Quality, safety and processing of wild and farmed fish

edited by

J.B. Luten
C. Jacobsen
K. Bekaert
A. Sæbø
J. Oehlenschläger

Wageningen Academic
P u b l i s h e r s

ISBN: 978-90-8686-005-0
e-ISBN: 978-90-8686-581-9
DOI: 10.3920/978-90-8686-581-9

Cover design: Oddvar Dahl, Fiskeriforskning
Cover photo: Frank Gregersen, Fiskeriforskning

First published, 2006

Foreword

The route from fish to the final product ready to be eaten by the consumer is a long one. Therefore product quality, safety and processing have always been important issues in seafood research.

However, over the last years seafood research has undergone a number of changes. Integration of various scientific disciplines in former traditional technological oriented seafood research has taken place. The importance of consumer science in the seafood research area has increased. Most consumers consider fish as healthy and nutritious. In spite of this, some European countries experience a trend towards declining seafood consumption. It is therefore crucial to understand consumer behaviour and well-being to seafood and to adapt seafood products to consumer demands.

Scientific and technological developments in the field of food have led to a marked shift in the way consumers deal with food and health. There is a growing awareness that the dietary source and form of food may affect the overall health of the consumer. In case of seafood there are established roles of vitamin D and calcium in bone health promotion and of omega-3 poly-unsaturated fatty acids (PUFA) in reducing the risk of cardiovascular disease. At the same time, PUFA also pose a great challenge to food and fish technologists. This is due to the fact that PUFA are very susceptible to oxidation due to their polyunsaturated nature. Thus, to maintain the healthy properties of the PUFA, lipid oxidation must be prevented in fish products and fish oil enriched foods. Scientifically based knowledge about processing technology and lipid chemistry is necessary to obtain this goal.

Provision of seafood from capture fish is declining and partly not sustainable. Seafood from aquaculture can potentially overcome this problem. It can deliver a product of defined quality and composition to the market in all seasons of the year enabling a greater penetration of 'healthy foods' in the diet of consumers. With increasing intensification the ability to determine the quality of the product emerges in several ways, which lends itself to tailor-made seafood products. In addition, high seafood quality should be linked to ethically acceptable husbandry practices and aquaculture systems, in reality and as perceived by the consumers. Furthermore, it is important to diversify farming to various white fish species, such as cod and carp. However, these 'new species' are likely to be more susceptible to quality problems. Therefore new knowledge about quality of farmed fish is essential.

In 2005 the unique opportunity was taken to combine two important international meetings in the seafood research area. The Institute for Marine Resources & Ecosystem Studies (IMARES, former RIVO)) and the Unit Animal Sciences – Fisheries (D-VI, former SFD) from the Belgium Institute for Agricultural and Fisheries Research were the organising host institutes for 35th annual meeting of the West European Fish Technologists Association (WEFTA). Karen Bekaert (D-VI) and my self were in charge of the organisation. The decision to combine this event with the yearly meeting of the European section of the American Oil Chemists Society was initiated by Charlotte Jacobsen from the Danish Institute for Fisheries Research.

In this combined meeting we decided to focus on quality, safety and seafood consumer's issues and to give room for presentations from aquaculture. In this way we were able to cover the scientific topics in the total seafood chain area from fish to dish.

With this book the editorial team is aiming to reach a broad group of readers, from students, experienced scientists to actors in the seafood chain. In the eight chapters of this book scientists from various disciplines address the advances in seafood research with respect to quality, safety, consumer's demand and processing of wild and farmed fish. Each chapter contains applied research papers and research notes. Many papers are a reflection from on-going national and European collaborative projects. Experts have refereed all papers. The editorial team of this book wish to express their gratitude for their referee work.

My special thanks go to my co-editors Charlotte Jacobsen, Karen Bekaert, Asgeir Sæbø (Natural Lipids LtD AS, Norway) and Jörg Oehlenschläger (Federal Research Centre for Nutrition and Food, Germany). The two intensive editorial working days of Charlotte, Karen, Jörg and I in Madrid showed the good team-spirit we have had from the beginning to realise this book.

I would like to thank my two colleagues at Fiskeriforksning, Tromsø, Norway: Oddvar Dahl for his creative work to design the cover of the book and Frank Gregersen for providing the photos for the cover.

Mieke van der Putte (IMARES) is thanked for her help in collecting all the papers.

Last but not least my thanks to the management of Fiskeriforskning for giving me the opportunity to realise this book here in the beautiful Northern part of Norway within the context of my combined job at Fiskeriforskning and IMARES

Joop Luten

Fiskeriforskning, Tromsø, Norway
IMARES, IJmuiden, The Netherlands

Table of contents

Chapter 1:
Nutritional properties and oxidation of marine lipid

Seafood is a very important source of healthy lipids. Particularly the long chain omega-3 fatty acids have been shown to have numerous health effects in the human body. At the same time, marine lipids also pose a great challenge to food and fish technologists. This is due to the fact that omega-3 fatty acids are very susceptible to oxidation due to their polyunsaturated nature. Thus, to maintain the healthy properties of the omega-3 fatty acids, lipid oxidation must be prevented in fish products and fish oil enriched foods. Scientifically based knowledge about processing technology and lipid chemistry is necessary to obtain this goal.

This chapter covers several of the aspects mentioned above. For example, the nutritional properties of marine phospholipids originating from krill are discussed together with possible nutritional and technological applications of marine phospholipids. The effect of using so-called structured lipids containing omega-3 fatty acids on the plasma and liver levels of triglycerides and cholesterol is reported in another paper. A third paper concerns the level of cholesterol in seafood, which has been determined in a very large number of fish and shellfish.

The possibilities of enriching foods with omega-3 fatty acids with the aim of increasing the consumer's intake of these healthy fatty acids are reported in two papers. One dealing with fish oil enriched mayonnaise and the other dealing with fish oil enriched yoghurt.

Protection against lipid oxidation by the addition of antioxidative compounds to fish oil, liposomes or fish products is reported in a number of different papers. Promising results with natural compounds such as rosemary extract, tea catechins, tocopherol and grape fibres are presented. In addition, synergistic effects between tocopherol and ascorbate are shown in another paper. Finally, both pro-oxidative and antioxidative effects of ascorbate in salted cod muscle are also presented.

When evaluating lipid oxidation, it is necessary to use reliable and reproducible methods. A wide range of standard methods have been available for many years. Recently developed methods include fluorescence spectroscopy for measuring TBARS and electron spin resonance (ESR) for measuring free radicals. Two papers discuss the applicability of these two methods and the pitfalls for the ESR method is also dealt with.

Marine phospholipids (MPL): Resources, applications and markets

Erik Løvaas
University of Tromsø, Department of Pharmacy, N-9037 Tromsø, Norway

Abstract

Marine phospholipids (MPL) are extremely valuable components that can be applied within diverse areas like nutrition, pharmacy, medicine and basic research. MPL can be extracted from diverse marine raw material, in particular from roe and krill but also from by-products from the fishery industry. This review describes the chemical characteristics of marine phospholipids, their natural resources, and some present and future applications. The possible market size for MPL is estimated by using the omega-3 market as an indicator.

Keywords: marine phospholipids, omega-3 fatty acids, nutraceuticals, pharmaceuticals, antioxidants

Introduction

There is a current interest in omega-3-lipids with a high content of EPA (C20:5 n-3) and DHA (C22:6 n-3). The interest is based on the observations that omega-3 fatty acids have a number of interesting biological actions, like anti-inflammatory effects (James and others 2000; Ross 1999; Adam 2003), prevention of cardiovascular diseases (Siscovick and others 2000; Marckmann and others 1999; Anonymous 1999), improved neuronal function (Loudes and others 1983), learning behaviour (Wainwright 2000; Zimmer and others 2000; Uauy and others 1996) and visual function (Horrocks and others 1999; Jacobson 1999), retardment of cognitive impairment in old people (Kalmijn and others 1997; Bourre 2004; Haag 2003; Horrocks and others 1999; Markesbery 1997; Youdim and others 2000), and preventive effects of breast (Bartsch and others 1999) and prostate cancer (Leitzmann and others 2004). There is evidence to suggest that omega-3 LCPUFA (long chain polyunsaturated fatty acids), DHA in particular, increase the sensitivity of cancer cells to anticancer agents and pro-oxidants (Timmer-Bosscha and others 1998; Germain and others 1998).

The demand for polyunsaturated fatty acids in health related products is increasing (Anonymous 2005), and there are ongoing efforts to identify methods for supplying fish oils in forms, which are compatible with established consumer habits and consumer trends. Late developments have been to produces soft capsules and to produce microencapsulated oils that can be mixed with dry material (like flour) and used in composite products (like bread).

Phospholipids extracted from certain marine organisms have a high content of omega-3-fatty acids, with EPA and DHA as main components. Marine phospholipids have potential applications in human and animal nutrition, in pharmacology and in drug delivery. Liposomes made of marine phospholipids may also serve as convenient targets for oxidation processes, and can be used for high throughput screening of antioxidants.

Characteristics of marine phospholipids (MPL)

Phospholipids are the building blocks of biological membranes, and constitute the border between the internal milieu of the cell and the external environment. Phospholipids are the most fundamental prerequisite for organizing matter into living, self-reproducing entities. When phospholipids are mixed with water they spontaneously aggregate into micro-spheres (liposomes) where a phospholipid membrane encloses an internal space (Lasic and Papahadjopoulos 1998). Phospholipids have numerous applications, some related to their physical properties, and some to their biochemical properties.

Phospholipids are formed from four individual components: (1) fatty acids, (2) a negatively charged phosphate group, (3) an alcohol and (4) a backbone (Figure 1). There is variability in the alcohol component and in the fatty acid components, giving rise to a great number of different phospholipid species. The biggest variability is in the fatty acid components.

Phospholipids have a hydrophilic head and a hydrophobic tail. The hydrophobic tail tends to aggregate itself in non-polar associations, while the hydrophilic head group forms stable interactions in polar (aqueous) environments. When phospholipids are introduced into an aqueous environment they spontaneously form nano-structures (liposomes). Liposomes are versatile structures that are applied as delivery vehicles (*e.g.* site specific drug delivery agents). They are also realistic models for biological membranes. In particular one should note that fatty acids attached to a phospholipid are "water soluble".

The term "Marine Phospholipids" is used to describe a subgroup of phospholipids that has a high content of marine omega-3 fatty acids. Such phospholipids are abundant in marine organisms. In particular they are present in high concentrations in krill and in fish roe. They are also present in high amounts in the human brain (Svennerholm 1968; Clandinin 1999; Erren and others 2004) and in specific cell membranes (Uauy and others 2001).

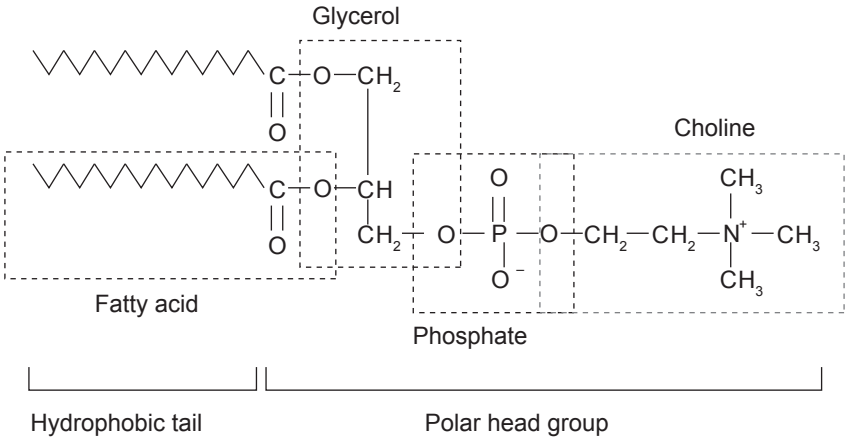


Figure 1. Structure of a phospholipid.

For many years there have been efforts to produce phospholipids with a high content of omega-3 fatty acids. One method has been to force feed hens with marine oils, and subsequently isolate the phospholipids from the egg yolk (Surai and others 2000). Such drastic methods generates phospholipids that maximum contain a few percent of omega-3 fatty acids. In contrast, marine phospholipids from roe typically contain 50% omega-3 fatty acids (Figure 2). The omega-3 fatty acids are generally esterified in the 2-position of the phospholipid (Kuksis 1972; Amate and others 1999).

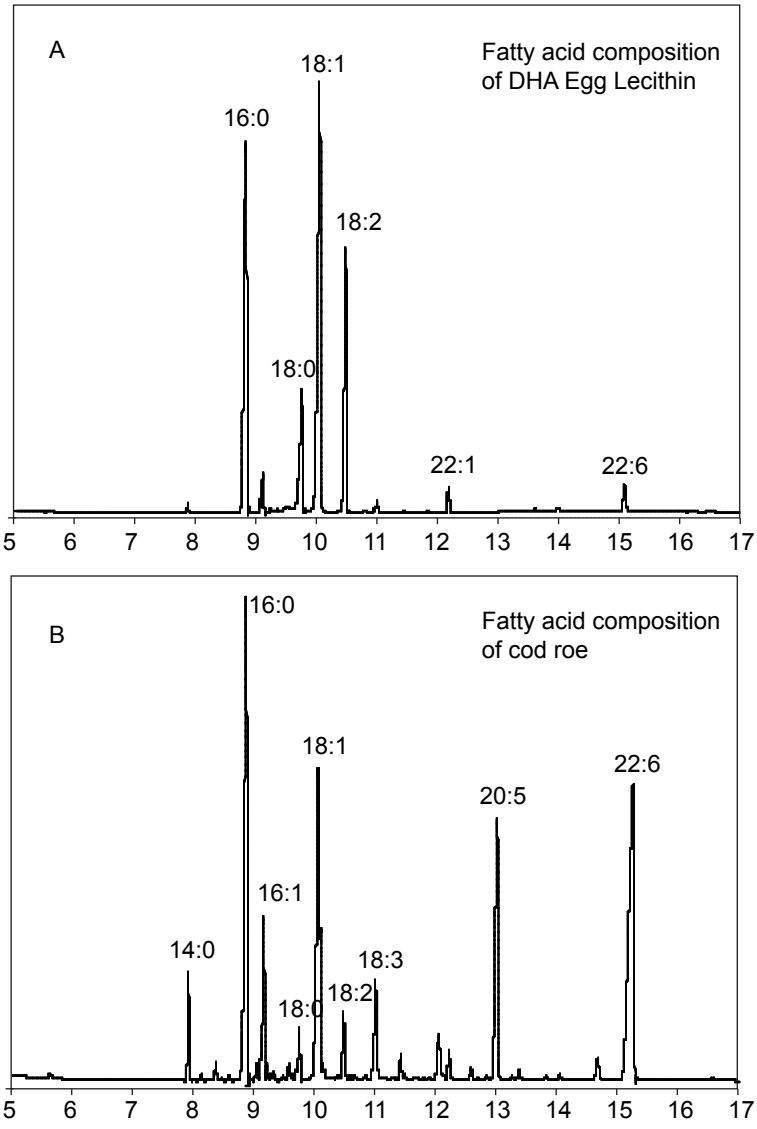


Figure 2. Fatty acid profile of (A) DHA egg Lecithin, and (B) marine phospholipids from cod roe.

Natural resources of MPL

Marine phospholipids can be isolated from a variety of raw materials. Current raw materials include krill, fish roe and fish meal, which all have characteristic properties making them more or less suitable for phospholipid extraction. Important factors to consider when choosing a raw material are:

- Price of raw material
- Level of marine phospholipids in raw material
- Quality and freshness of raw material
- Catch volume and stability of catch over an extended period
- Available process technology
- Pollutants in raw material
- Laws and regulations (*e.g.* relating to "novel food")

Antarctic krill *(Euphausia superba)* is a small shrimp like zooplankton (crustacean) that plays a key role in the Antarctic food web. Krill is one of the biggest biological resources on earth. The biomass of Antarctic krill is estimated to 1.350 million tons, about five times the weight of the human population (Stevens 1995). Krill swarms can occupy an area of 500 sq km, and hold up to 20,000 individuals per cubic meter. Biomass densities attain 10 – 16 kg/m^3. Unlike other Euphausiid species, *Euphausia superba* feeds almost exclusively upon phytoplankton. This contributes to the very high abundance and potential catch of krill, since there are comparatively small losses between the primary production and the potential harvest by man. Krill occurs in groups or large swarms and occupies a niche similar to that of the herring in the North Atlantic, since large schools of pelagic fish are absent. They attain a size of 7 cm (normal range 4.5 – 5.0 cm) and feed primarily on phytoplankton or sea ice algae.

The catch limit on krill in the South Atlantic Region is set to 4 million tons (Hewitt and others 2004), but the annual krill catch has never amounted to more than 550,000 tons (Dommarsnes and others 2004; Anonymous 2006). It is still the largest crustacean fishery in the world. The catch is by trawls with small mesh size and big openings, and catch efficiency can be 5 – 20 tons hour. The price for krill is approx. 700 US$/ton delivered at the Falkland Islands. Transport from South America to Canada or North Europe is about 150 US$/ton.

There is a considerable variation in the amount of lipid and the lipid composition of krill. The lipid content may vary between 12.5 and 0.5% (w/w wet mass) with about 25% as phosphatidyl choline (Pond and others 1995). Specific production technologies give rise to a phospholipid enriched oil, containing as much as 40 – 60% phospholipids, of which phosphatidyl choline is the dominating (>75%) species. The phospholipids have a high level of omega-3 fatty acids (approx. 40%), and the EPA:DHA ratio is 25:15 (which makes it similar to 18:12 oil from South America). Such oils are used as dietary supplements.

Recently roe has been identified as a good source for marine phospholipids. Developments on process technology will also make fish meal attractive in the future.

Applications of MPL

Marine phospholipids have potential applications in diverse areas like nutraceuticals (functional food, dietary supplements, sports nutrition, maternal nutrition, and baby food), pharmaceuticals

(liposomes – site specific drug delivery, clinical nutrition), cosmeceuticals (liposomes), and biochemistry (model membranes, liposomal test systems for antioxidants).

The word "potential" is used deliberately, because many of the mentioned applications have not been tested at the present time. Marine phospholipids are new at the market place, and their range of applications has yet to be determined. However, intensive development work is going on, and it is probable that soon a number of new products will become available.

Test system for antioxidants

The best-documented applications of MPL are related to liposomes. Liposomes made of MPL have been developed as a test system for antioxidants, and as model systems for oxidation of biological membranes. Some basic principles for these developments will be reviewed here.

Liposomes made from marine phospholipids oxidize fast in the presence of trace amounts of Fe^{2+}, and the oxidation can be followed by UV/VIS spectroscopy.

A summary of the assay is presented in Figure 3a and is reviewed by Barstad (2006). Basically, the liposomes are prepared by mixing MPL and lipid soluble antioxidants in a common organic solvent (*e.g.* methanol:chloroform). The ratio of antioxidant to phospholipid is typically 1:100 (mol:mol). Buffer is added after removal of the solvent under a stream of nitrogen, and the phospholipids are allowed to swell at ambient temperature for 60 minutes. Shaking the solution produce milky white multilamellar vesicles (MLV) (Chatterjee and others 1988), which are sonicated to give small unilamellar vesicles (SUV). Liposome oxidation is then initiated by addition of a catalytic

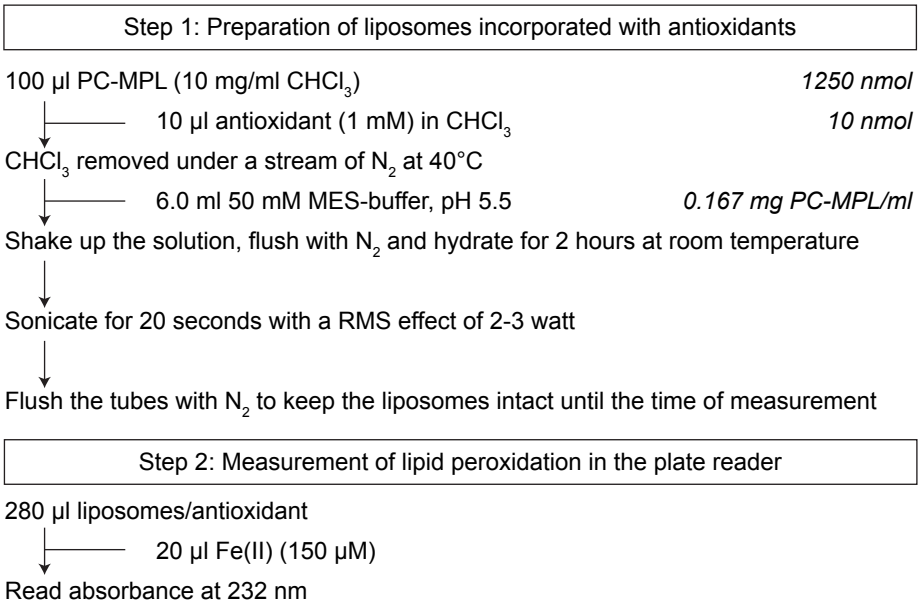


Figure 3a. Outline of procedures for the liposome antioxidant assay.

amount of Fe^{2+}, and the oxidation is monitored by following the development of dienes (UV/VIS measurements at 232 nm). By doing the assay in a plate reader it is possible to investigate a great number of samples at the same time. As an example is shown the simultaneous testing of 13 antioxidants, all added in the same concentration (Figure 3b).

The system has a number of merits that makes it an attractive method for screening of new compounds for antioxidative effects:

- It is easy to prepare liposomes that have lipid soluble test compounds incorporated into a membrane. The test system does not experience the same problems as do for example LDL, where lipid soluble antioxidants are difficult to incorporate.
- Liposomes are realistic models for biological membranes, and thus represent a test system which is more likely to pick up antioxidative effects which are relevant in medicine and pharmacology.
- Antioxidative effects can be measured in true time as formation of dienes can be measured by UV-spectroscopy. No derivatization is necessary, or any need to manipulate samples prior to measurement. This increases the reliability of the method, and makes it useful as a routine screening assay.
- Antioxidants are incorporated into the membrane in a predetermined stoichiometry. By manipulating the fluidity of the membrane the effect of dynamic movements of the membrane components (lipids and antioxidants) can be studied. Detailed information on the mechanistic relationships can thus be obtained.
- Liposomes represent an interphase where synergistic effect of water-soluble and lipid soluble antioxidants conveniently can be studied. The system is well suited for studying complex relationships between antioxidative compounds.
- Oxidation is initiated by addition of a catalytic amount of initiator (Fe^{2+}), and a free radical process is initiated. Different types of antioxidants (including both metal chelators and free radical scavengers) retard the oxidation process in a concentration dependent fashion. The extent and mode of inhibition is characteristic of the antioxidant under investigation.

Nutraceutical applications

There is an increasing attention on marine phospholipids because of their potential nutritional value and their unique biophysical properties. Central to this interest is the fact that enzymes that are involved in lipid metabolism (lipases) essentially acts in an aqueous environment. Water soluble lipases have better access to water-soluble substrates (like phospholipids) than to water insoluble substrates (like triglycerides). In sum this means that omega-3 fatty acids from marine phospholipids are more easily accessible for catabolic processes than triglycerides.

The present imbalance in the fatty acid intake represent serious trouble for the health situation in the general population, and there is a strong desire to improve the situation by introducing new products on the market with a high level of omega-3 fatty acids and low level of omega-6 fatty acids. Numerous studies have demonstrated that omega-3 fatty acids have positive effects on preventing or curing diseases like heart disease, cancer, rheumatoid arthritis, diabetes, ulcerative colitis, allergies, eczema, skin thickening, weight gain and a host of other diseases. Researchers are now also linking inadequate intake of these omega-3 fats in pregnant women to premature birth and low birth weight (Al and others 2000; Crawford 2000; Koletzko 2002; Smuts and others 2003), and to hyperactivity in children (Burgess and others 2000).

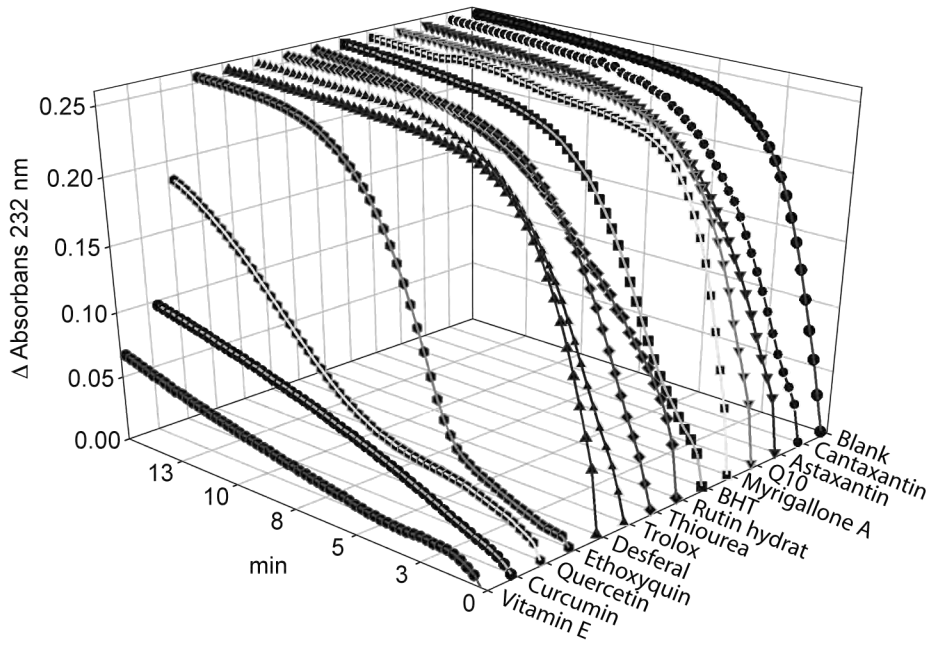


Figure 3b. Simultaneous testing of antioxidants by the liposome diene conjugation assay. The assay is run at room temperature (25 °C).

Pregnancy nutrition and infant formula

Important brain growth occurs during pregnancy and the first few years of life, and there are several nutritional building blocks that are vital for brain growth and visual function (Bryan and others 2004; Smuts and others 2003; Lauritzen and others 2001; Crawford 2000; Neuringer 2000; Uauy and others 2000; Carlson 1999). The most important of these are omega-3 fatty acids (DHA) and omega-6 fatty acids (arachidonic acid, or ARA). Many expectant mothers are not aware that what they eat makes a substantial difference in the health of their unborn child.

The DHA content of breast milk depends on the mother's diet. Most pregnant women have a low intake of omega-3 fat, which ultimately may have consequences for the unborn and the suckling child. It is thus advised that the expectant mother should eat fish several times a week to increase brain building DHA, or alternatively that dietary supplements should be considered when fish intake is not sufficient. Marine phospholipids should be likely candidates for such products.

There has long been a concern that lipids in infant formulas may be absorbed to a lesser extent than breast milk. It has been suggested that absorption of fish oil in pre-term infants may be poor as the amount of DHA required in formulas was almost a magnitude greater than usually provided by human milk (Liu and others 1987). A low absorption means that more lipids are left in the intestine, giving rise to adverse effects. In particular more triacylglycerols (TAG) and

fatty acids will appear in the colon and lead to conditions like intestinal obstruction, increase in colon fatty acid soap content, and altered microbial composition (Ling and others 1997; Verkade and others 1991).

Addition of nutritional marine phospholipids to infant formula may have several advantageous effects in neonates. Firstly, there is evidence that TAG absorption may be impaired when the supply of exogenous PL is insufficient for micelle formation during fat absorption (Levy and Roy 2005). Secondly, nutritional phospholipids would only require the action of phospholipase A_2 on one fatty acid to produce free fatty acids and 1-lysophospholipid that would be readily absorbed, utilizing more of the dietary lipid, leaving fewer lipids left in the intestine. In premature infants it has been found that DHA from egg PL was better absorbed than DHA from breast milk and TAG from single cell sources. Improved absorption of lipid and long chain PUFA from egg phospholipids has also been shown in other studies (Amate and others 2001; Makrides and others 2002). It was also found that giving omega-3 enriched eggs (presumably high contents of PL DHA) to infants of 6-12 months of age increased erythrocyte DHA concentration to 30-40% above that of infants fed breast milk and can be used to maintain a high omega-3 PUFA level. Phospholipids are also required for intestinal lipoprotein formation and lipid distribution outside the enterocytes (Tso and others 1984). Lack of nutritional PL could lead to impaired enterocyte lipoprotein synthesis causing lipid accumulation in the cells.

Omega-3 and the brain

Though the brain is able to make some of what it needs, the brain is surprisingly dependent upon raw materials from the diet, notably fatty acids (Bryan and others 2004; Finley and others 2001; Lauritzen and others 2001; Innis 1994). If the right fat is not supplied, brain structure and brain function is altered. The brains requirement for highly specified fats, coupled with the poor dietary choices of most people living on modern diets, has created a problem in our society. Particular interest has been ascribed to omega-3 fatty acids. Recent findings show the positive effects of these fatty acids on:

- Bipolar depression. It is known that EPA and DHA gives a significant reduction in the symptoms of depression (Logan 2004)
- Age-related memory loss and Alzheimer's disease. It has been shown that adults with dementia, Alzheimer's and cognitive problems have a low level of DHA in the blood, and it is suggested that deficiency of these fatty acids in the diet may be a risk factor for cognitive impairment (Bryan and others 2004; Markesbery 1997; Calon and others 2005; Morris and others 2003)
- Reading and learning problems in children. Children with fatty acid deficiency have been found to have poorer reading abilities (Burgess and others 2000; Hals and others 2000).
- Intelligence in children. Breastfed children were compared with formula fed children who had none of the fatty acid DHA in their formula. The breast fed group had a significant higher IQ score at 8 years of age (Koo 2003).

It is highly desirable to produce food ingredients that meet the needs of the consumers by having an attractive texture and flavour profile and good oxidation stability, and at the same time having a healthy composition.

Market trends for MPL (human nutrition)

The market for MPL is still in its infancy. However, an increasing activity in the field is observed, and a number of companies are preparing market introduction of either natural MPL, derivatives of natural MPL, or synthetic MPL. The development of entirely new market segments, as well as a penetration into existing markets on omega-3 products is expected. It seems reasonable to assume that the MPL market will follow the general trends of omega-3 fish oils.

The global market for omega-3 fatty acids is estimated to 15-20,000 tons, derived from a total world production of fish oil of approximately 300,000 tons/year. Of this 10-12,000 tons are refined fish oils, 6-7,000 tons cod liver oil, 1000 tons tuna oil, and 300 tons DHA/algae oil. About 1.500 tons of the market is concentrates with 50-70% omega-3 fatty acids, and a variable ratio between EPA and DHA (Anonymous 2005; Anonymous 2003). A market share of 1% equals a demand of 150 tons marine phospholipids/year.

The nutraceutical industry has identified "health" as one of the top 10 "mega trends" in the years to come. The industry on a worldwide basis is presently valued to US$ 7.1 billion/year, and is growing by 6.1% annually (Anonymous 2005). In North America and in Europe, fish oils are the fastest growing supplement, with an estimated annual growth rate between 15 and 18%. From 1994, where the sales were approximately $27 million, to $190 million in 2003, the overall growth was an impressive 603% (Anonymous 2003).

Conclusion

There is a search for new products. Marine phospholipids have a range of biochemical and biophysical properties that is attractive for many applications and market segments, and certainly new products that will find applications in food and pharma will be developed. At present there is a need to scale up production of these compounds and to identify natural resources that comply with regulatory issues. The business prospects for this class of compounds will undoubtedly attract attention from a diverse group of actors, including producers, distributors, pharmaceutical companies, researchers, and investors.

References

Adam O. 2003. Dietary fatty acids and immune reactions in synovial tissue. Eur J Med Res 8(8):381-387.

Al MDM, Van Houwelingen AC, Hornstra G. 2000. Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. Am J Clin Nutr 71(1):285S-291S.

Amate L, Ramírez M, Gil A. 1999. Positional analysis of triglycerides and phospholipids rich in long-chain polyunsaturated fatty acids. Lipids 34(8):865-871.

Amate L, Gil A, Ramirez M. 2001. Feeding infant piglets formula with long-chain polyunsaturated fatty acids as triacylglycerols or phospholipids influences the distribution of these fatty acids in plasma lipoprotein fractions. J Nutr 131 (4):1250-1255.

Anonymous. 1999. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 354 (9177):447-455.

Anonymous. 2003. Rapport nr. 4613/111: Internasjonal markeds- og industrianalyse for marine ingredienser. RUBIN Trondheim, Norway.

Anonymous. 2005. Report No. 1818: World Nutraceuticals. The Fredonia Group Inc Cleveland, USA.

Anonymous. 2006. Statistical Bulletin (1995 - 2004). (17), CCAMLR Hobart, Australia.

Barstad H, Alvik AC, Løvaas E. 2006. Antioxidant synergy effect betweenn α-tocopherol and ascorbate on the autoxidation of liposomes. In: Luten JB, Jacobsen C, Bekaert K, Sæbø A, Oehlenschläger J, editors. Seafood research from fish to dish: Quality, safety, and processing of wild and farmed fish. Wageningen: Wageningen Academic Publishers. pp.87-94.

Bartsch H, Nair J, Owen RW. 1999. Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers. Carcinogenesis 20(12):2209-2218.

Bourre JM. 2004. Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing. J Nutr Health Aging 8(3):163-174.

Bryan J, Osendarp S, Hughes D, Calvaresi E, Baghurst K, van-Klinken JW. 2004. Nutrients for cognitive development in school-aged children. Nutr Rev 62(8):295-306.

Burgess JR, Stevens L, Zhang W, Peck L. 2000. Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder. Am J Clin Nutr 71(1):327S-330S.

Calon F, Lim GP, Morihara T, Yang F, Ubeda O, Salem NJ, Frautschy SA, Cole GM. 2005. Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. Eur J Neurosci 22(3):617-626.

Carlson SE. 1999. Long-chain polyunsaturated fatty acids and development of human infants. Acta Paediatrica Supplement 88:72-77.

Chatterjee SN, Agarwal S. 1988. Liposomes as membrane model for study of lipid peroxidation. Free Radic Biol Med 4:51-72.

Clandinin MT. 1999. Brain development and assessing the supply of polyunsaturated fatty acid. Lipids 34(2):131-137.

Crawford MA. 2000. Placental delivery of arachidonic and docosahexaenoic acids: implications for the lipid nutrition of preterm infants. Am J Clin Nutr 71(1):275S-284S.

Dommarsnes A, Iversen SA, Løbach T. 2004. Fiskerier i Antaktis. Havets ressurser 2004 160-163. Havforskningsinsituttet Bergen, Norway.

Erren TC, Erren M. 2004. Can fat explain the human brain's big bang evolution? Horrobin's leads for comparative and functional genomics. Prostaglandins Leukot Essent Fatty Acids 70(4):345-347.

Finley JW, Shahidi F. 2001. The chemistry, processing, and health benefits of highly unsaturated fatty acids. An overview. ACS Symposium Series 788:2-11.

Germain E, Chajes V, Cognault S, Lhuillery C, Bougnoux P. 1998. Enhancement of doxorubicin cytotoxicity by polyunsaturated fatty acids in the human breast tumor cell line MDA-MB-231: relationship to lipid peroxidation. Int J Cancer 75(4):578-583.

Haag M. 2003. Essential fatty acids and the brain. Can J Psychiatry 48(3):195-203.

Hals J, Bjerve KS, Nilsen H, Svalastog AG, Ek J. 2000. Essential fatty acids in the nutrition of severely neurologically disabled children. Br J Nutr 83:219-225.

Hewitt RP, Watkins J, Naganobu M, Sushin V, Brierley AS, Demer D, Kasatkina S, Takao Y, Goss C, Malyshko A. 2004. Biomass of Antarctic krill in the Scotia Sea in January/February 2000 and its use in revising an estimate of precautionary yield. Deep Sea Research Part II: Topical Studies in Oceanography 51(12-13):1215-1236.

Horrocks LA, Yeo YK. 1999. Health benefits of docosahexaenoic acid (DHA). Pharmacol Res 40:211-225.

Innis SM. 1994. The 1993 Borden award lecture. Fatty acid requirements of the newborn. Can J Physiol Pharmacol 72:1483-1492.

Jacobson SW. 1999. Assessment of long-chain polyunsaturated fatty acid nutritional supplementation on infant neurobehavioral development and visual activity. Lipids 34(2):151-160.

James MJ, Gibson RA, Cleland LG. 2000. Dietary polyunsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr 71(1 Suppl):343S-348S.

Kalmijn S, Feskens EJM, Launer LJ, Kromhout D. 1997. Polyunsaturated fatty acids, antioxidants, and cognitive function in very old men. Am J Epidemiol 145:33-41.

Koletzko B. 2002. Parenteral lipid infusion in infancy: physiological basis and clinical relevance. Clin Nutr 21:53-65.

Koo WW. 2003. Efficacy and safety of docosahexaenoic acid and arachidonic acid addition to infant formulas: can one buy better vision and intelligence? J Am Coll Nutr 22(2):101-107.

Kuksis A. 1972. Newer developments in determination of structure of glycerides and phosphoglycerides. Progr Chem Fats Lipids 12:1-163.

Lasic D, Papahadjopoulos D. 1998. Medical Applications of Liposomes. Amsterdam: Elsevier. 779p.

Lauritzen L, Hansen HS, Jørgensen MH, Michaelsen KF. 2001. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 40(1-2):1-94.

Leitzmann MF, Stampfer MJ, Michaud DS, Augustsson K, Colditz GC, Willett WC, Giovannucci EL. 2004. Dietary intake of n-3 and n-6 fatty acids and the risk of prostate cancer. Am J Clin Nutr 80(1):204-216.

Levy E, Roy CC. 1989. Developmental aspects of intestinal lipoprotein synthesis and secretion In: Lebenthal M, editor. Human Gatrointestinal Development. New York: Raven Press. p 491-502.

Ling SC, Weaver IT. 1997. The fate of fat in the infant's colon. Q J Med 90:553-555.

Liu CC, Carlson SE, Rhodes PG, Rao VS, Meydrech EF. 1987. Increase in plasma phospholipid docosahexaenoic and eicosapentaenoic acids as a reflection of their intake and mode of administration. Pediatr Res 22(3):292-296.

Logan AC. 2004. Omega-3 fatty acids and major depression: A primer for the mental health professionals. Lipids Health Disease 3(25):1-8.

Loudes C, Faivre-Bauman A, Barret A, Grouselle D, Puymirat J, Tixier-Vidal A. 1983. Release of immunoreactive TRH in serum-free cultures of mouse hypothalamic cells. Brain Res 285(2):231-234.

Makrides M, Hawkes JS, Neumann MA, Gibson RA. 2002. Nutritional effect of including egg yolk in the weaning diet of breast-fed and formula-fed infants: a randomized controlled trial. Am J Clin Nutr 75(6):1084-1092.

Marckmann P, Grønbæk M. 1999. Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. Eur J Clin Nutr 53(8):585-590.

Markesbery WR. 1997. Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med 23:134-147.

Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, Aggarwal N, Schneider J. 2003. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 60(7):940-946.

Neuringer M. 2000. Infant vision and retinal function in studies of dietary long-chain polyunsaturated fatty acids: methods, results, and implications. Am J Clin Nutr 71(1):256S-267S.

Pond D, Watkins J, Priddle J, Sargent J. 1995. Variation in the lipid content and composition of Antarctic krill Euphausia superba at South Georgia. Mar Ecol Prog Ser 117:49-57.

Ross R. 1999. Atherosclerosis is an inflammatory disease. Am Heart J 138(5):S419-S420.

Siscovick DS, Raghunathan TE, King I, Weinmann S, Bovbjerg VE, Kushi L, Cobb LA, Copass MK, Psaty BM, Lemaitre R, Retzlaff B, Knopp RH. 2000. Dietary intake of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. Am J Clin Nutr 71(1):208S-212S.

Smuts CM, Borod E, Peeples JM, Carlson SE. 2003. High-DHA eggs: Feasibility as a means to enhance circulating DHA in mother and infant. Lipids 38(4):407-414.

Stevens JE. 1995. Dismantling the Myths of the Southern Ocean: The Secret Lives of Krill. Sea Frontiers 20(2):26-31.

Surai PF, MacPherson A, Speake BK, Sparks N-HC. 2000. Designer egg evaluation in a controlled trial. Eur J Clin Nutr 54(4):298-305.

Svennerholm L. 1968. Distribution and fatty acid composition of phosphoglycerides in normal human brain. J Lipid Res 9(5):570-579.

Timmer-Bosscha H, de-Vries EG, Meijer C, Oosterhuis JW, Mulder NH. 1998. Differential effects of all-trans-retinoic acid, docosahexaenoic acid, and hexadecylphosphocholine on cisplatin-induced cytotoxicity and apoptosis in a cisplantin-sensitive and resistant human embryonal carcinoma cell line. Cancer Chemother Pharmacol 41(6):469-476.

Tso P, Drake DS, Black DD, Sabesin SM. 1984. Evidence for separate pathways of chylomicron and very low-density lipoprotein assembly and transport by rat small intestine. Am J Physiol 247(6):G599-G610.

Uauy R, Peirano P, Hoffman D, Mena P, Birch D, Birch E. 1996. Role of essential fatty acids in the function of the developing nervous system. Lipids 31 Suppl:S167-S176.

Uauy R, Hoffman DR. 2000. Essential fat requirements of preterm infants. Am J Clin Nutr 71(1):245S-250S.

Uauy R, Mena P. 2001. Lipids and neurodevelopement. Nutr Rev 59(8 Pt 2):S34-S46.

Verkade HJ, Hoving EB, Muskiet FA, Martini IA, Jansen G, Okke A, Vonk RJ, Bijleveld CM. 1991. Fat absorption in neonates: comparison of long-chain-fatty-acid and triglyderide compositions of formula, feces, and blood. Am J Clin Nutr 53:643-651.

Wainwright P. 2000. Nutrition and behaviour: the role of n-3 fatty acids in cognitive function. Br J Nutr 83(4):337-339.

Youdim KA, Martin A, Joseph JA. 2000. Essential fatty acids and the brain: Possible health implications. Internat J Develop Neurosci 18(4-5):383-399.

Zimmer L, Delpal S, Guilloteau D, Aioun J, Durand G, Chalon S. 2000. Chronic n-3 polyunsaturated fatty acid deficiency alters dopamine vesicle density in the rat frontal cortex. Neurosci Lett 284(1-2):25-28.

Effects of dietary triacylglycerol structure on plasma and liver lipid levels in rats fed low-fat diets containing n-3 polyunsaturated fatty acids of marine origin

Trine Porsgaard[1], Xuebing Xu[2] and Huiling Mu[1]
[1]Biochemistry and Nutrition Group, [2]Food Biotechnology and Engineering Group, BioCentrum-DTU, Technical University of Denmark, 2800 Lyngby, Denmark

Abstract

For three weeks rats were fed low-fat diets of which 25% was n-3 polyunsaturated fatty acids (PUFAs). The n-3 PUFAs originated either from two structured triacylglycerols (TAGs) differing in intramolecular structure or from fish oil. A group fed chow was included as control. Dietary inclusion of n-3 PUFAs increased the level of n-3 PUFAs in plasma and liver. TAG structure influenced the content of individual n-3 PUFAs meaning that enrichment of the *sn*-2 position with a specific fatty acid increased the level of this fatty acid. Differences in dietary TAG structure did not influence plasma and liver levels of TAG and cholesterol.

Keywords: cholesterol, n-3 polyunsaturated fatty acids, rats, structured lipids, triacylglycerols

Introduction

Marine oils contain n-3 polyunsaturated fatty acids (PUFAs), especially 20:5n-3 and 22:6n-3. The *sn*-2 position of the glycerol moiety in fish oil is enriched with PUFAs, especially 22:6n-3, whereas in oils of marine mammals, including whale and seal oil, this is reversed, *i.e.* there is less PUFA enrichment in the *sn*-2 position (Ackman 1988). A high intake of n-3 PUFAs has been associated with a beneficial effect on the plasma lipid profile, leading to a low incidence of coronary heart disease (Kris-Etherton and others 2002). Furthermore, n-3 PUFAs were shown to influence the brain and visual development during infancy (Uauy and others 2001) and to have immunomodulating effects (Yaqoob 2003). In addition, positive effects of fish oil have been reported on mental health (Peet and Stokes 2005) and in patients with rheumatoid arthritis (Kremer 2000).

In this paper the term structured triacylglycerols (TAGs) will cover TAGs that have been modified or restructured from natural oils and fats, and from fatty acids for specific nutritional purposes. Structured TAGs can be produced by chemical modification or enzyme technology (Xu 2000). By using enzymatic modification with lipases having specific selectivity, specific structured TAGs can be produced, whereas randomized TAGs may be produced both by chemical and enzymatic modification. TAGs containing both medium-chain fatty acids (MCFAs) and long-chain fatty acids (LCFAs) on the same glycerol molecule combine the advantages of the easily digested and absorbed MCFAs with the delivery of various PUFAs with specific effects in the body. TAGs with this structure (MLM-type, M=MCFA, L=LCFA) were shown to improve the absorption of essential fatty acids during malabsorption (Hubbard and Mckenna 1987; Christensen and others 1995b; Tso and others 1999; Rubin and others 2000; Straarup and Høy 2000) and to increase the uptake of PUFAs for tissue regeneration after surgery (Kenler and others 1996; Kruimel and others 2001).

Studies have shown that the absorption of dietary TAGs in the immediate absorptive phase depends on the intramolecular structure with enhanced absorption of fatty acids located in the *sn*-2 position (Ikeda and others 1991; Christensen and others 1995a; Straarup and Høy 2000; Porsgaard and others 2005b). This results from the preferential hydrolysis by the pancreatic lipase in the digestive tract of fatty acids located in the *sn*-1,3 positions resulting in *sn*-2 monoacylglycerols (*sn*-2 MAGs) and free fatty acids (FFA) (Mattson and Volpenhein 1964). Studies investigating the longer-term effects of structured TAGs have shown that the intramolecular structure might influence plasma and liver lipid profiles in rats (Nagata and others 2003, 2004), while no differences in amounts of 22:6n-3 in brain phospholipids of rat pups from dams fed different dietary TAG structures and n-3 sources were observed (Hartvigsen and others 2004).

The aim of the present study was to compare the effects of differences in dietary TAG structure on liver and plasma lipid levels of TAG and cholesterol, and on the fatty acid compositions, when n-3 PUFAs were included in the dietary oils. Due to the many positive results reported with n-3 PUFAs, it is of outmost importance to investigate if the effects of n-3 PUFAs are influenced by their position on the glycerol backbone. For this purpose, two structured TAGs containing n-3 PUFAs and differing in intramolecular structure were produced by enzymatic interesterification and included as part of low-fat diets for rats, while two other groups were fed fish oil or ordinary rat chow. After 3 weeks feeding, the contents of TAG and cholesterol in plasma and livers were measured together with the fatty acid compositions.

Materials and methods

Diets

Two specific structured TAGs, LML (L=LCFA, M=MCFA) and MLM, were produced by lipase-catalysed interesterifications as described previously (Porsgaard and others 2005a). Safflower oil was from Urtekram A/S (Mariager, Denmark), olive oil from FDB (Albertslund, Denmark), and fish oil from Aarhus United A/S (Aarhus, Denmark). The overall composition of the diets containing LML, MLM, or fish oil was as follows (in wt %): 56 corn starch (Bestfoods Nordic A/S, Skovlunde, Denmark); 20 casein (Miprodan milk proteins, Arla Foods amba, Viby, Denmark), 10 sucrose (Danisco Sugar, Copenhagen, Denmark), 5 salt mixture (including trace elements), 4 fat, 4 cellulose powder (MN 100, Machery-Nagel GmbH & Co, Düren, Germany), 0.5 vitamin mixture, and 0.5 choline chloride (Merck, Darmstadt, Germany). The vitamin and salt mixtures were composed as described by Aaes-Jørgensen and Hølmer (1969).

The diets were balanced with respect to content of saturated, monounsaturated, n-6, and n-3 PUFA. The LML diet contained 53% LML, 21.5% safflower oil, and 25.5% olive oil; the MLM diet 52% MLM, 21.5% safflower oil, and 26.5% olive oil; and the fish oil diet 79% fish oil and 21% safflower oil. The diets were powdered, stored at –20 °C and provided fresh every day. One group of rats was fed ordinary pelleted rat chow (Altromin No. 1324, Chr. Petersen A/S, Ringsted, Denmark) with 4 wt % fat. All diets and water were supplied *ad libitum*.

Animals

Thirty-two male specific-pathogen free Wistar rats (Taconic M & B A/S, Ll. Skensved, Denmark) were included in the study. They were housed two per plastic cage in a temperature (21 °C) and humidity (50%) controlled environment on a 12 hour – 12 hour light/dark cycle. They were acclimatized to the housing conditions for 10 days, in which they all were fed the rat chow

diet. The rats were then randomised to the four dietary groups for the following 3 weeks. At the end of the feeding period, rats were fasted over-night, killed by decapitation, blood was collected in heparin glasses and the livers were excised, weighed, and immediately frozen in liquid nitrogen. Plasma isolated by centrifugation and livers were stored at -80 ºC until analysis. The experiment was approved by the Danish Committee for Animal Experiments.

Lipid analyses

Lipid from diets, plasma, and livers were extracted with chloroform and methanol (Folch and others 1957). Fatty acid composition of total TAG and the structure of TAG represented by the fatty acid composition in *sn*-2 MAG of dietary fats were measured as described previously (Porsgaard and Høy 2000). Lipid extracts from plasma and liver were methylated with a borontriflourid (BF_3) catalysed method (Morrison and Smith 1964) and analysed by gas-chromatography using the following procedure: a Hewlett-Packard 5890 chromatograph was equipped with a split/splitless injector, SP2380 capillary column (60 m, I.D. 0.25 mm; Supelco Inc., Bellefonte, PA), flame-ionisation detection and helium as carrier gas. Conditions were as follows: injector temperature 270 °C, flame ionisation detector 270 °C, helium carrier gas at 1.2 ml/min, injector split ratio 1:11. Initial oven temperature was 70 °C which was held for 5 min followed by temperature programming: 15 °C/min until 160 °C followed by 1.5 °C/min until 200 °C, which was maintained for 15 min and finally the temperature was raised to 225 °C and maintained for 10 min. Peak areas were calculated using a Hewlett-Packard computing integrator. The fatty acids were identified by comparing the retention time with standards of known fatty acid composition.

Plasma TAG and cholesterol were measured enzymatically using commercial kits (Roche Diagnostics GmbH, Mannheim, Germany). Liver TAG and cholesterol contents were measured by quantitative high-performance thin layer chromatography (HPTLC) using a slight modification of a method described by Müller and others (2004). Briefly, livers added cholesteryl acetate (Sigma, St. Louis, MO) during extraction as internal standard were redissolved in a defined volumen of chloroform/methanol (95/5, vol/vol). Samples and a quantitative standard mixture composed of cholesterol, triolein, and cholesteryl acetate (a six-point standard curve was applied on each plate) were applied on prewashed and activated silica HPTLC plates (Silica Gel-60, Merck GmbH, Darmstadt, Germany) using a Desage AS-30 HPTLC applicator (Desage GmbH, Wiesloch, Germany). The HPTLC plates were developed in a Camag Horizontal TLC-chamber (Camag, Muttenz, Switzerland) in the first run in a solvent system consisting of hexane/diethyl ether/formic acid (80/20/1, vol/vol/vol) and in the second run in heptane/diethyl ether (95/5, vol/vol) increasing the length of the development by 2 cm in comparison with the first run. Following development, the plates were soaked with charring reagent (633 mM $CuSo_4 \cdot 5H_2O$ in 8% phosphoric acid) and heated at 160 ºC for 8 min. Quantification was performed through densitometry at 420 nm, using a Desaga CD-60 HPTLC densitometer in reflectance mode. R^2 values for the regression lines were never below 0.99.

Statistics

Results are expressed as mean values with their standard errors of the mean (SEM), n=8. Differences between groups were tested by one-way ANOVA (GraphPad PRISM version 3.02, GraphPad software, San Diego, CA). Tukey's Multiple Comparison post test was used to determine which means differed. Differences were considered significant at $p<0.05$.

Results and discussion

Dietary fats

Fatty acid compositions of the dietary fat mixtures, shown in Table 1, were balanced with respect to content of saturated (24.2-26.5 wt %), monounsaturated (30.4-34.6 wt %), n-6 PUFAs (18.0-20.6 wt %), and n-3 PUFAs (22.9-24.9 wt %). In contrast, the chow diet was enriched in 18:2n-6 and with a low content of n-3 PUFAs. The content of 20:5n-3 was higher in the MLM and LML diets than in the fish oil diet and the content of 22:6n-3 was higher in the LML and fish oil diets than in the MLM diet, but overall the n-3 PUFA contents in the diets were comparable. In the LML diet, the *sn*-2 position of dietary TAGs was enriched in 10:0, whereas the MLM diet was enriched in 20:5n-3 in the *sn*-2 position, and the fish oil diet in 22:6n-3. We used low-fat diets (4 wt %) with fat contents similar to ordinary rat chow in contrast to comparable studies where higher dietary fat loads were given (10 wt %) (Nagata and others 2003, 2004). On the other hand, the structured TAGs used in the studies by Nagata and others were purified by preparative high-performance liquid chromatography leading to a higher purity of the structured TAGs. The cost of making highly purified structured TAGs is too high for using the oils as commercial products, but they are of scientific value when studying absorption parameters. In the present study, we chose to use non-purified structured TAGs leading to the presence of other TAG species than the desired ones, but with a relative enrichment of the MLM and LML species in the two oils, respectively.

Body weight gains and liver weights of rats

The rats weighed 249 ± 2 g at the beginning of the experiment and gained on average 81 ± 3, 82 ± 6, 85 ± 3, and 73 ± 7 g in the LML, MLM, fish oil, and chow groups, respectively (no differences between groups). After 3 weeks feeding, liver weights were 8.9 ± 0.3, 8.8 ± 0.4, 8.7 ± 0.2, and 7.9 ± 0.4 g in the LML, MLM, fish oil, and chow groups, respectively (no differences between groups). Thus, weight gain and final liver weights were not influenced by dietary treatment.

TAG and cholesterol contents of plasma and liver

The highest plasma TAG concentration after 3 weeks feeding (Figure 1A) was measured in the fish oil group (1.3 ± 0.1 mmol/l) and it was significantly higher than in the chow group (0.8 ± 0.2 mmol/l, $p<0.05$). Parallel to the plasma TAG levels, the highest liver TAG content (Figure 2A) was measured in the fish oil group (33.8 ± 5.9 mg/g liver) and it was significantly higher than in the chow group (17.2 ± 1.6 mg/g liver, $p<0.05$). No differences were measured in either plasma or liver TAG concentrations between the three groups fed n-3 PUFAs. In many studies, fish oil was shown to lower plasma TAG concentrations both in humans (reviewed by Kris-Etherton and others 2002) and in some animal species, including rats, in comparison with lard (Otto and others 1992), compared with corn oil (Herman and others 1991; Kim and others 2004), or with coconut and safflower oils (Mohan and others 1991). Some studies showed greater plasma TAG lowering effect the higher the dietary fish oil concentration (Bourre and others 1990; Nieuwenhuys and others 1998). On the contrary, Willumsen and others (1993) showed that 22:6n-3 in contrast to 20:5n-3 did not possess a hypotriglyceridemic effect when fed as highly purified free fatty acids to rats. In our experiment, the highest 22:6n-3 contents were found in fish oil and LML and could partly explain that the highest plasma TAG concentrations were found in these groups, although only statistically significant difference between the fish oil and chow groups was found. Another factor that might have influenced the results was that the effects of a non-purified diet (chow) were compared with the effects of purified diets.

Table 1. Fatty acid composition of total TAG and in the sn*-2 position of dietary fats (wt %)*[1].

FA	LML total TAG	MLM	Fish oil	Chow	LML *sn*-2 position	MLM	Fish oil	Chow
10:0	16.1	10.8	2.9	-[2]	32.2	2.5	1.5	-
14:0	0.5	1.2	4.6	0.2	0.7	1.9	7.4	0.3
16:0	6.1	8.5	13.6	17.4	2.0	5.6	15.4	3.8
16:1n-7	0.5	1.4	4.0	0.2	0.6	1.8	4.5	0.3
18:0	3.1	3.1	2.7	2.8	1.6	2.5	1.4	0.5
18:1n-9	25.9	24.7	16.0	17.4	30.1	28.9	15.3	19.9
18:1n-7	1.6	2.1	1.9	1.2	1.1	2.5	1.3	0.5
18:2n-6	17.0	19.3	17.1	54.2	20.6	22.2	20.2	68.8
18:3n-3	0.6	0.9	2.1	5.5	0.9	1.2	2.1	5.8
18:4n-3	0.5	1.8	3.0	-	0.1	2.0	3.1	-
20:0	0.5	0.4	0.2	0.3	0.3	0.3	0.1	-
20:1n-9	1.4	1.9	5.0	0.4	1.1	2.0	2.0	0.2
20:4n-6	0.8	1.0	0.5	-	0.2	1.0	0.5	-
20:4n-3	0.6	0.7	0.6	-	0.1	0.9	0.6	-
20:5n-3	13.0	14.0	8.2	0.2	4.3	17.0	9.4	-
22:1n-11	0.8	2.0	7.5	-	0.6	1.7	1.9	-
22:5n-3	1.8	0.8	0.7	-	0.7	1.1	1.1	-
22:6n-3	8.3	4.8	8.6	0.1	1.2	4.5	11.7	-
Others[3]	0.9	0.6	0.8	0.1	1.6	0.4	0.5	-
SFA[4]	26.5	24.2	24.2	20.7	37.3	12.9	26.1	4.6
MUFA[4]	30.4	32.3	34.6	19.2	34.5	37.0	25.0	20.9
n-6 PUFA[4]	18.2	20.6	18.0	54.5	21.0	23.5	21.0	68.8
n-3 PUFA[4]	24.9	22.9	23.2	5.7	7.2	26.6	27.9	5.8

[1] Values are the mean of three determinations.
[2] A dash (-) means not detected.
[3] Others represent fatty acids that contributed less than 0.5 wt % of total fatty acids.
[4] SFA, MUFA, n-6 PUFA, and n-3 PUFA represent the sum of total saturated, monounsaturated, n-6 polyunsaturated, and n-3 polyunsaturated fatty acids, respectively.

Feeding of non-purified diets versus semi-purified diets was previously found to influence the atherogenicity in hamsters (Nicolosi and others 1998). Furthermore, we used low-fat diets in contrast to most other studies that have been performed with medium or high dietary fat loads (Bourre and others 1990; Mohan and others 1991; Herman and others 1991; Otto and others 1992). A factor that could also influence the outcome of the experiment.

The plasma cholesterol concentrations after 3 weeks feeding (Figure 1B) were between 1.4 ± 0.3 and 2.0 ± 0.2 mmol/l in rats from the 4 dietary groups (no differences between groups). In livers, the cholesterol contents (Figure 2B) were between 2.7 ± 0.3 and 3.5 ± 0.4 mg/g liver (no differences between groups). In humans, dietary fish oil has limited effect on plasma cholesterol contents (reviewed by Kris-Etherton and others 2002), while in rats serum cholesterol levels were decreased in some experiments (Mohan and others 1991; Otto and others

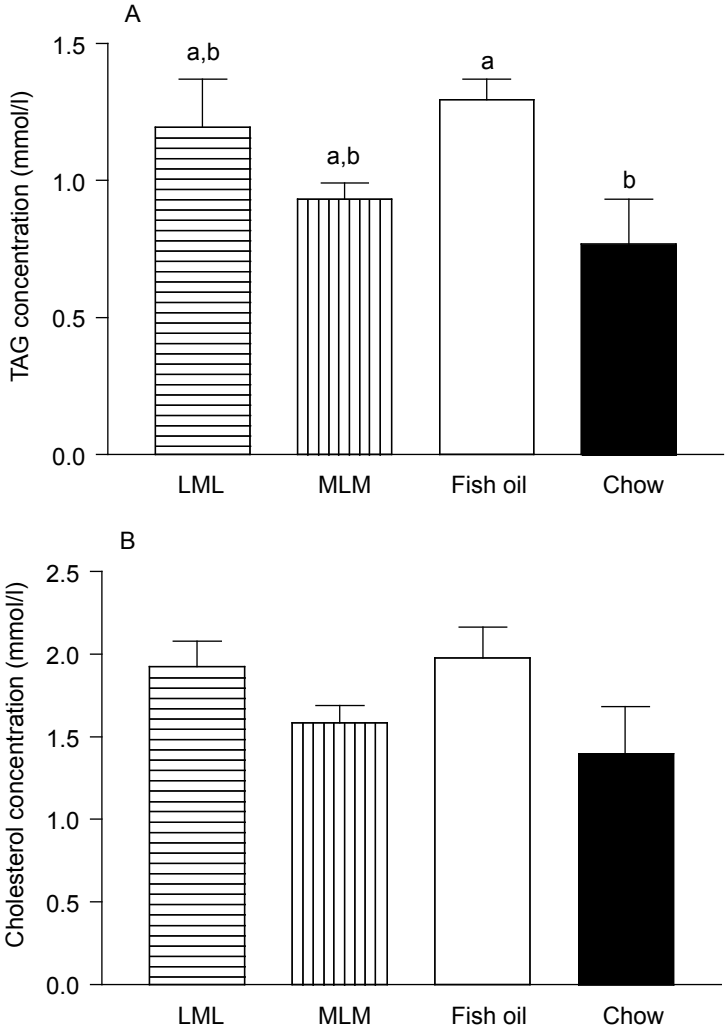


Figure 1. Plasma concentrations of TAG (A) and cholesterol (B) (mmol/l) in rats following 3 weeks feeding (LML, MLM, fish oil, and chow dietary groups). Data are mean ± SEM of 8 rats. Values not sharing a common letter are significantly different (P<0.05).

1992; Nieuwenhuys and others 1998), or the effect depended on the background diet (Herman and others 1991).

In the present study, TAG structure had no influence on plasma and liver TAG and cholesterol concentrations. This is in contrast to the studies by Nagata and others (2003, 2004). With highly purified structured TAGs of the MLM- and LML-types where L was 18:2n-6 and M either 8:0 or 10:0 (purity 90-92%), serum TAG levels were lower in rats fed LML-type TAGs than in those fed MLM-type TAGs (Nagata and others 2003). All structured TAGs decreased serum cholesterol compared with the control corn oil with a tendency for lower levels in serum from

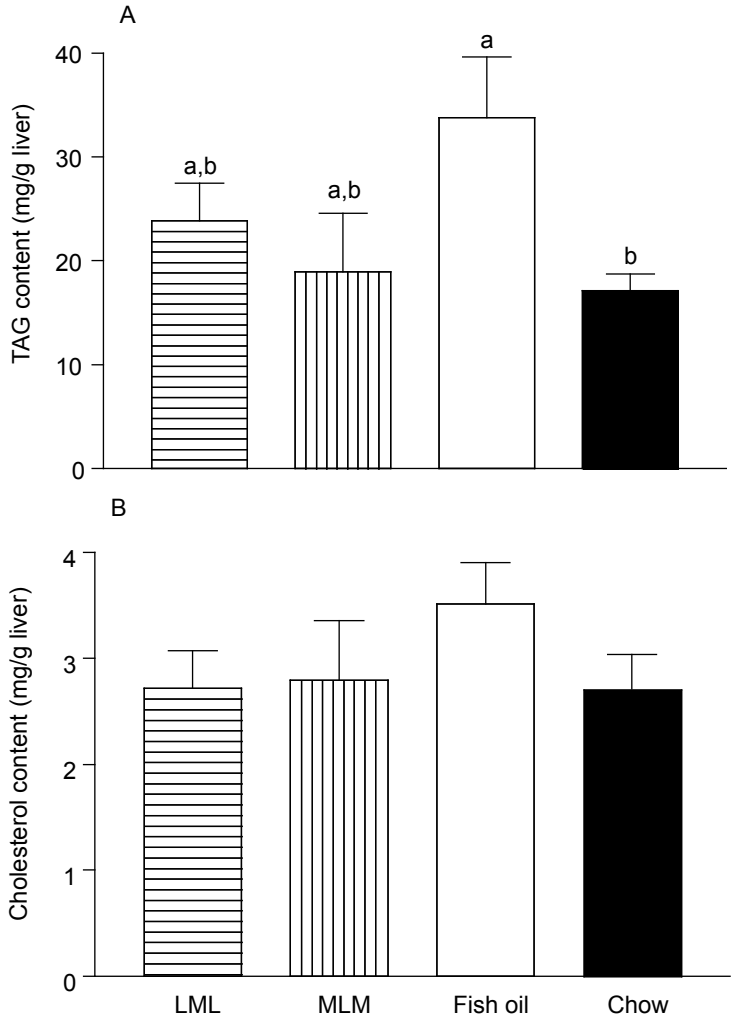


Figure 2. Liver contents of TAG (A) and cholesterol (B) (mg/g liver) in rats following 3 weeks feeding (LML, MLM, fish oil, and chow dietary groups). Data are mean ± SEM of 8 rats. Values not sharing a common letter are significantly different (P<0.05).

rats fed TAGs containing 10:0 in comparison with those fed 8:0. No differences were observed in liver cholesterol levels, while liver TAG levels were lower in rats fed TAGs containing 10:0 than in those fed corn oil. When purified TAGs of the structure MML or MLM, where M was 8:0 and L was either 20:5n-3 or 22:6n-3 (purity 68-82%) was fed to rats, all structured TAGs led to lower serum cholesterol, TAG, and phospholipid levels than the control soybean oil (Nagata and others 2004). Minor differences were observed between the structured TAGs with the highest levels observed in the 22:6n-3/8:0/8:0 fed rats. In hamsters fed either fish or seal oil, serum TAG and cholesterol levels were not influenced by differences in dietary TAG structure, while

liver cholesterol levels were highest and TAG levels were lowest after fish oil feeding where the *sn*-2 position of the glycerol moiety in fish oil was enriched with n-3 PUFAs (Yoshida and others 2001). In rats fed seal or squid oil no effects of differences in dietary TAG structure were observed on plasma and liver TAG and cholesterol levels (Ikeda and others 1998). On the basis of these studies, no overall conclusion can be drawn regarding the effect of dietary intramolecular TAG structure on levels of TAG and cholesterol in plasma and liver. Factors like purity of dietary structured TAGs, dietary fat levels and fatty acid compositions, and species may influence the results.

Fatty acid composition of plasma and liver

No 10:0 was detected in either plasma or liver of rats fed MLM and LML (Table 2 and 3) indicating that this MCFA was either oxidized in the liver or metabolized to a longer-chain derivative. Previous studies have shown that some MCFAs are absorbed through the lymphatic system (Porsgaard and others 2005b; Straarup and others 2005) with decreasing absorption the shorter the chain length of the MCFA (Mu and Høy 2000), but the major part of MCFAs is absorbed through the portal vein for further oxidation in the liver (Bernard and Carlier 1991).

Table 2. Fatty acid composition of plasma from rats (wt %)[1].

	LML		MLM		Fish oil		Chow	
FA	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:0	0.6	$0.0^{a,b}$	0.5	$0.0^{a,b}$	0.6	0.1^{a}	0.4	0.0^{b}
16:0	22.4	0.3^{a}	22.2	0.3^{a}	23.0	0.2^{a}	20.6	0.4^{b}
16:1n-7	2.8	0.1^{a}	2.9	0.2^{a}	3.3	0.5^{a}	1.3	0.1^{b}
18:0	6.2	0.4^{b}	6.4	$0.3^{a,b}$	6.0	0.5^{b}	8.0	0.5^{a}
18:1n-9	11.6	0.6^{a}	11.1	0.3^{a}	10.6	0.2^{a}	7.2	0.5^{b}
18:1n-7	2.4	0.1^{a}	2.4	0.1^{a}	2.6	0.1^{a}	2.1	0.1^{b}
18:2n-6	19.3	$0.5^{b,c}$	18.6	0.5^{c}	21.8	0.7^{b}	25.5	0.9^{a}
18:3n-3	0.4	0.0^{b}	0.4	0.0^{b}	0.8	0.1^{a}	1.0	0.1^{a}
20:4n-6	14.8	0.9^{b}	16.0	0.5^{b}	12.2	0.7^{b}	30.0	1.7^{a}
20:5n-3	9.8	0.5^{b}	11.7	0.5^{a}	8.0	0.6^{b}	0.7	0.0^{c}
22:5n-3	1.6	0.1^{b}	2.1	0.1^{a}	1.4	0.1^{b}	0.4	0.1^{c}
22:6n-3	7.5	0.2^{b}	5.4	0.2^{c}	9.3	0.2^{a}	2.7	0.1^{d}
Others[2]	0.6	0.0	0.3	0.0	0.4	0.0	0.1	0.0
SFA[3]	29.1	0.5	29.1	0.4	29.6	0.6	29.0	0.5
MUFA[3]	16.8	0.7^{a}	16.4	0.5^{a}	16.5	0.6^{a}	10.6	0.6^{b}
n-6 PUFA[3]	34.5	0.7^{b}	35.0	0.8^{b}	34.3	0.8^{b}	55.6	0.9^{a}
n-3 PUFA[3]	19.5	0.5^{a}	19.6	0.6^{a}	19.5	0.9^{a}	4.8	0.2^{b}

[1]Values represent the mean ± SEM of 8 rats in each group. Values in rows not sharing a common superscript letter were significantly different ($P<0.01$).

[2]Others represent fatty acids that contributed less than 0.5 wt % of total fatty acids.

[3]SFA, MUFA, n-6 PUFA, and n-3 PUFA represent the sum of total saturated, monounsaturated, n-6 polyunsaturated, and n-3 polyunsaturated fatty acids, respectively.

Table 3. Fatty acid composition of total lipids from rat livers (wt %)[1].

	LML		MLM		Fish oil		Chow	
FA	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
16:0	20.2	0.3^{a,b}	19.6	0.3^{b,c}	20.8	0.3^{a}	19.0	0.3^{c}
16:1n-7	2.2	0.1^{b}	2.5	0.2^{b}	3.5	0.1^{a}	0.9	0.1^{c}
18:0	11.3	0.4^{b}	10.4	0.5^{b}	9.9	0.3^{b}	13.5	0.7^{a}
18:1n-9	7.8	0.5^{a}	7.1	0.2^{a}	7.9	0.3^{a}	5.3	0.5^{b}
18:1n-7	2.6	0.1^{b}	2.6	0.1^{b}	3.0	0.1^{a}	2.4	0.1^{b}
18:2n-6	16.6	0.3^{b}	16.7	0.3^{b}	18.3	0.6^{b}	23.3	0.7^{a}
18:3n-3	0.3	0.0^{b}	0.3	0.0^{b}	0.7	0.1^{a}	0.7	0.1^{a}
20:3n-6	0.6	0.0^{a,b}	0.6	0.0^{b}	0.7	0.0^{a}	0.3	0.0^{c}
20:4n-6	15.9	0.7^{b,c}	17.8	0.6^{b}	13.9	0.6^{c}	28.1	1.0^{a}
20:5n-3	8.2	0.6^{b}	10.6	0.7^{a}	7.0	0.4^{b}	0.4	0.0^{c}
22:5n-3	2.3	0.1^{b}	2.9	0.2^{a}	1.8	0.1^{c}	0.8	0.0^{d}
22:6n-3	11.3	0.3^{a}	8.7	0.2^{b}	12.0	0.3^{a}	4.7	0.3^{c}
Others[2]	0.7	0.1	0.2	0.0	0.5	0.1	0.6	0.1
SFA[3]	31.9	0.6^{a,b}	30.4	0.4^{b}	31.3	0.5^{a,b}	32.9	0.6^{a}
MUFA[3]	12.6	0.6^{a,b}	12.2	0.5^{b}	14.4	0.4^{a}	8.7	0.6^{c}
n-6 PUFA[3]	33.1	0.6^{b}	35.0	0.9^{b}	32.9	0.8^{b}	51.8	0.6^{a}
n-3 PUFA[3]	22.4	0.8^{a}	22.4	0.8^{a}	21.4	0.6^{a}	6.6	0.3^{b}

[1]Values represent the mean ± SEM of 8 rats in each group. Values in rows not sharing a common superscript letter were significantly different (P<0.01).
[2]Others represent fatty acids that contributed less than 0.5 wt % of total fatty acids.
[3]SFA, MUFA, n-6 PUFA, and n-3 PUFA represent the sum of total saturated, monounsaturated, n-6 polyunsaturated, and n-3 polyunsaturated fatty acids, respectively.

In plasma, the major differences were observed in fatty acid compositions between rats fed n-3 PUFAs and rats fed chow. The higher content of n-6 PUFAs in the chow diet was reflected in plasma leading to significantly higher contents of 18:2n-6, 20:4n-6, and total n-6 PUFA in the chow group and lower contents of 16:0, 16:1n-7, 18:1n-9, 18:1n-7, 20:5n-3, 22:5n-3, 22:6n-3, total monounsaturated fatty acids, and total n-3 PUFAs compared with the other three groups (p<0.01). In liver, similar differences were observed between the groups fed n-3 PUFA and the chow group. This means that the dietary fatty acid composition was reflected both in plasma and in liver. Furthermore, the dietary TAG structure was reflected in the plasma and liver fatty acids. MLM that was enriched in 20:5n-3 in the *sn*-2 position of TAG led to a higher content of this fatty acid, while fish oil, which was enriched in 22:6n-3 in the *sn*-2 position, resulted in the highest amount of this fatty acid in plasma and liver. A higher content of 22:5n-3 was found both in plasma and in livers of rats fed MLM in comparison with the other n-3 PUFA diets. This fatty acid was partly provided by the diet and partly formed through elongation of 20:5n-3 originating from enrichment of the *sn*-2 position in the MLM oil. Although the individual n-3 PUFAs in plasma and liver differed between the three groups fed dietary n-3 PUFAs, the overall n-3 PUFA content was similar as were the overall contents of saturated, monounsaturated, and n-6 PUFAs. Åkesson and others (1978) found that approximately 25% of the fatty acid initially located in the *sn*-2 position of dietary fat had migrated to the *sn*-1,3 positions during hydrolysis

and lymphatic absorption. This implies a general conservation of approximately 75% of the fatty acids located in the *sn*-2 position, which is important when considering the possible advantages in tailor-making fats with particular TAG structures and maintaining the location of fatty acids in specific positions following absorption. From the present study, it seems that enrichment of specific LCFAs in the *sn*-2 position of dietary fat will lead to a higher level of that specific fatty acid in the organs; thus tailor-making is indeed possible.

Conclusion

From the results presented in this study, it can be concluded that dietary TAG structure influenced fatty acid composition of livers and plasma, but had no influence on the concentrations of TAG and cholesterol, when fed as part of low-fat diets to rats. The structured TAGs used in the present study contained MCFAs and n-3 PUFAs of marine origin and were produced by enzymatic interesterification without purification.

Acknowledgements

We greatly appreciate the technical help of Karen Jensen and Jannie Agersteen. Lillian Vile and Nina Kjeldsen are thanked for taking care of the rats. Financial support was obtained from The Danish Technological Research Council.

References

Aaes-Jørgensen E, Hølmer G. 1969. Essential fatty acid-deficient rats: I. Growth and testes development. Lipids 4(6):501-506.

Ackman RG. 1988. Some possible effects on lipid biochemistry of differences in the distribution on glycerol of long-chain *n*-3 fatty acids in the fats of marine fish and marine mammals. Atherosclerosis 70(1-2):171-173.

Bernard A, Carlier H. 1991. Absorption and intestinal catabolism of fatty acids in the rat: effect of chain length and unsaturation. Exp Physiol 76(3):445-455.

Bourre JM, Bonneil M, Dumont O, Piciotti M, Calaf R, Portugal H, Nalbone G, Lafont H. 1990. Effect of increasing amounts of dietary fish oil on brain and liver fatty composition. Biochim Biophys Acta 1043(2):149-152.

Christensen MS, Høy C-E, Becker CC, Redgrave TG. 1995a. Intestinal absorption and lymphatic transport of eicosapentaenoic (EPA), docosahexanoic (DHA), and decanoic acids: dependence on intramolecular triacylglycerol structure. Am J Clin Nutr 61(1):56-61.

Christensen, MS, Müllertz A, Høy C-E. 1995b. Absorption of triglycerides with defined or random structure by rats with biliary and pancreatic diversion. Lipids 30(6):521-526.

Folch J, Lees M, Stanley GHS. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226(1):497-509.

Hartvigsen MS, Mu H, Hougaard KS, Lund SP, Xu X, Høy C-E. 2004. Influence of dietary triacylglycerol structure and level of n-3 fatty acids administered during development on brain phospholipids and memory and learning ability of rats. Ann Nutr Metab 48(1):16-27.

Herman S, Sediaoetama AD, Karyadi D, Beynen AC. 1991. Influence of background composition of the diet on the lipemic effect of fish oil *vs.* corn oil in rats. J Nutr 121(5):622-630.

Hubbard VS, McKenna MC. 1987. Absorption of safflower oil and structured lipid preparations in patients with cystic fibrosis. Lipids 22(6):424-428.

Ikeda I, Tomari Y, Sugano M, Watanabe S, Nagata J. 1991. Lymphatic absorption of structured glycerolipids containing medium-chain fatty acids and linoleic acid, and their effect on cholesterol absorption in rats. Lipids 26(5):369-373.

Ikeda I, Yoshida H, Tomooka M, Yosef A, Imaizumi K, Tsuji H, Seto A. 1998. Effects of long-term feeding of marine oils with different positional distribution of eicosapentaenoic and docosahexaenoic acids on lipid metabolism, eicosanoid production, and platelet aggregation in hypercholesterolemic rats. Lipids 33(9):897-904.

Kenler AS, Swails WS, Driscoll DF, DeMichele SJ, Daley B, Babineau TJ, Peterson MB, Bistrian BR. 1996. Early enteral feeding in postsurgical cancer patients. Fish oil structured lipid-based polymeric formula versus a standard polymeric formula. Ann Surgery 223(3):316-333.

Kim H-K, Choi S, Choi H. 2004. Suppression of hepatic fatty acid synthase by feeding α-linolenic acid rich perilla oil lowers plasma triacylglycerol level in rats. J Nutr Biochem 15(8):485-492.

Kremer JM. 2000. n-3 Fatty acid supplements in rheumatoid arthritis. Am J Clin Nutr 71(1 suppl):349S-351S.

Kris-Etherton PM, Harris WS, Appel LJ. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106(21):2747-2757.

Kruimel JW, Naber TH, van der Vliet JA, Carneheim C, Katan MB, Jansen JB. 2001. Parenteral structured triglyceride emulsion improves nitrogen balance and is cleared faster from the blood in moderately catabolic patients. J Parenteral Enter Nutr 25(5):237-244.

Mattson FH, Volpenhein RA. 1964. The digestion and absorption of triglycerides. J Biol Chem 239(9):2772-2777.

Mohan PF, Phillips FC, Cleary MP. 1991. Metabolic effects of coconut, safflower, or menhaden oil feeding in lean and obese Zucker rats. Br J Nutr 66(2):285-299.

Morrison WR, Smith LM. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 5:600-608.

Mu H, Høy C-E. 2000. Effects of different medium-chain fatty acids on intestinal absorption of structured triacylglycerols. Lipids 35(1):83-89.

Müller H, Hellgren LI, Olsen E, Skrede A. 2004. Lipids rich in phosphatidylethanolamine from natural gas-utililzing bacteria reduce plasma cholesterol and classes of phospholipids: a comparison with soybean oil. Lipids 39(9):833-841.

Nagata J, Kasai M, Watanabe S, Ikeda I, Saito M. 2003. Effects of highly purified structured lipids containing medium-chain fatty acids and linoleic acid on lipid profiles in rats. Biosci Biotechnol Biochem 67(9):1937-1943.

Nagata J, Kasai M, Negishi S, Saito M. 2004. Effects of structured lipids containing eicosapentaenoic or docosahexaenoic acid and caprylic acid on serum and liver lipid profiles in rats. BioFactors 22(1-4):157-160.

Nicolosi RJ, Wilson TA, Lawton C, Rogers EJ, Wiseman SA, Tijburg LBM, Kritchevsky D. 1998. The greater atherogenicity of nonpurified diets versus semipurified diets in hamsters is mediated via differences in plasma lipoprotein cholesterol distribution, LDL oxidative susceptibility, and plasma α-tocopherol concentration. J Nutr Biochem 9(10):591-597.

Nieuwenhuys CMA, Béguin S, Offermans RFG, Emeis JJ, Hornstra G, Heemskerk JWM. 1998. Hypocoagulant and lipid-lowering effects of dietary n-3 polyunsaturated fatty acids with unchanged platelet activation in rats. Arterioscler Thromb Vasc Biol 18(9):1480-1489.

Otto DA, Baltzell JK, Wooten JT. 1992. Reduction in triacylglycerol levels by fish oil correlates with free fatty acid levels in *ad libitum* fed rats. Lipids 27(12):1013-1017.

Peet M, Stokes C. 2005. Omega-3 fatty acids in the treatment of psychiatric disorders. Drugs 65(8):1051-1059.

Porsgaard T, Høy C-E. 2000. Lymphatic transport in rats of several dietary fats differing in fatty acid profile and triacylglycerol structure. J Nutr 130(6):1619-1624.

Porsgaard T, Kánský J, Mason S, Mu H. 2005a. Size and number of lymph particles measured by a particle sizer during absorption of structured oils in rats. Lipids 40(3):273-279.

Porsgaard T, Xu X, Göttsche J, Mu H. 2005b. Differences in the intramolecular structure of structured oils do not affect pancreatic lipase activity in vitro or the absorption by rats of (n-3) fatty acids. J Nutr 135(7):1705-1711.

Rubin M, Moser A, Vaserberg N, Greig F, Levy Y, Spivak H, Ziv Y, Lelcuk S. 2000. Structured triacylglycerol emulsion, containing both medium- and long-chain fatty acids, in long-term home parenteral nutrition: a double-blind randomized cross-over study. Nutrition 16(2):95-100.

Straarup EM, Høy C-E. 2000. Structured lipids improve fat absorption in normal and malabsorbing rats. J Nutr 130(11):2802-2808.

Straarup EM, Porsgaard T, Mu H, Hansen CH, Høy C-E. 2005. Lymphatic transport in rats of interesterified oils containing conjugated linoleic acids. Lipids 40(7):677-684.

Tso P, Lee T, DeMichele SJ. 1999. Lymphatic absorption of structured triglycerides *vs.* physical mix in a rat model of fat malabsorption. Am J Physiol 277(2 pt 1):G333-G340.

Uauy R, Hoffman DR, Peirano P, Birch DG, Birch EE. 2001. Essential fatty acids in visual and brain development. Lipids 36(9):885-895.

Willumsen N, Hexeberg S, Skorve J, Lundquist M, Berge RK. 1993. Docosahexaenoic acid shows no triglyceride-lowering effects but increases the peroxisomal fatty acid oxidation in liver of rats. J Lipid Res 34(1):13-22.

Xu X. 2000. Production of specific-structured triacylglycerols by lipase-catalyzed reactions: a review. Eur J Lipid Sci Technol 102(4):287-303.

Yaqoob P. 2003. Lipids and the immune response: from molecular mechanisms to clinical applications. Curr Opin Clin Nutr Metab Care 6(2):133-150.

Yoshida H, Ikeda I, Tomooka M, Mawatari M, Imaizumi K, Seto A, Tsuji H. 2001. Effect of dietary seal and fish oils on lipid metabolism in hamsters. J Nutr Sci Vitaminol 47(3):242-247.

Åkesson B, Gronowitz S, Herslof B, Ohlson R. 1978. Absorption of synthetic, stereochemically defined acylglycerols in the rat. Lipids 13(5):338-343.

Cholesterol content in seafood, data from the last decade: A review

Jörg Oehlenschläger
Federal Research Centre for Nutrition and Food, Department for Fish Quality, Palmaille 9, 22767 Hamburg, Germany

Abstract

An extensive review is given about the content of cholesterol in aquatic animals. Specimen of fish, crustaceans and molluscs have been collected onboard of the fishery research vessel "Walther Herwig III" and at local fish markets in the time period between 1992 and 2004. Most specimen are from North Atlantic waters (including Spitzbergen (Svalbard) and Greenland waters) some are of freshwater and brackish water origin. The data presented here are mainly on the cholesterol content in the edible part of the seafood (fish fillet, tail muscle and claw meat of crustaceans, whole mussels) but also about other parts of the animals used as a food item as the gonads (caviar, caviar preparations as spread). It is surprising how low the average cholesterol content in marine fish is (30 – 40 mg/kg). Furthermore, it is astonishing that the cholesterol content in edible part of marine fish (muscle) and in organs is not correlated with the respective lipid content. Contents in muscle of freshwater fish are somewhat higher, while the edible part of crustaceans and molluscs exhibit high cholesterol contents (up to over 200 mg/kg). Highest in cholesterol are fish eggs and food prepared from fish eggs as caviar and caviar derived products.

Keywords: cholesterol, marine fish, fishery products, crustaceans

Introduction

In this review cholesterol contents in edible part (fillet) and gonads of a number of aquatic species are reported. Data provided can help to improve the existing data in food composition tables and assist all those who have to eat a low cholesterol diet because of medical advice. This review will not deal with the physiological implications and metabolism of cholesterol in fish or in human beings.

Knowledge about the content of cholesterol in food in general and especially in fish and other types of seafood is of utmost importance for all those who have to take care for a reduction of cholesterol intake via food. Also for those who prepare foods or diets or who give recommendations about food intake profound knowledge about cholesterol content in the raw material used for food preparation is essential. This can be achieved effectively and reliable only when at least the average cholesterol content in fish and seafood based on a robust database is known, but even better when also information about its possible variation with season, catching ground and *e.g.* state of maturity is known. Furthermore, in fatty fish the interrelationship between fat content and cholesterol content must be known. Cholesterol content in processed fishery products can vary from the original cholesterol content present in the raw material due to fat and water processing losses, deterioration of cells and to some extent oxidation. The cholesterol molecule, however, is stable and is not degraded by the principal technological processes.

Unfortunately, there is still a lack in knowledge about cholesterol contents in fish and seafood. Food composition tables (*e.g.* Anon 1999; Souci, Fachmann and Kraut 2000; Piironen V and others 2002) show a lot of gaps and systematic investigations are rare or lacking completely. A limited number of reviews and contributions covering a broad number of species have been published (Feeley and others 1972; Kritchevsky and others 1967; Krzynowek 1985; Idler and Wiseman 1971; Teshima 1991; Kanazawa 2001; King and others 1990).

Since 1996 the occurrence and amount of cholesterol in the edible part of seafood is investigated quantitatively in the Department of Fish Quality of the Federal Research Centre for Nutrition and Food (formerly Institute for Biochemistry and Technology of the Federal Research Centre for Fisheries). A first report about the cholesterol content in lean fish species and in some crustaceans was given in Tromsö in 1997 during the 28th WEFTA meeting and a second later in Thessaloniki in 1998 during the 29th meeting (Oehlenschläger 1998). To broaden the basis of our knowledge these investigations have been continued and now new data about cholesterol content in gadoids, other seawater species, fatty pelagic species, flat fish species, crustacean and molluscan shellfish and other species can be presented.

Cholesterol is the main sterol in marine fish like haddock, pollock, salmon and in crustaceans like shrimp and lobster with over 90% of all sterols (Kritchevsky and others 1967). Oyster show only 41% cholesterol of sterols, crab 57% and scallop and clam 26% and 37%, respectively. A good review about the physiology and biochemistry of sterol in crustaceans, molluscs and fish can be found by Teshima (1991).

Cholesterol has been analysed in a number of seafood species from different areas of the world. Most of the investigations, however, have not been performed in a systematic way, do not cover more than one catching area and do not show seasonal variations in content. Ackman and McLeod (1988) have investigated cholesterol in fish and shellfish food products from Nova Scotia. They reported low cholesterol contents in ground fish and pelagic fish species, higher contents in molluscs and crustaceans (approx. 70-75 mg/100 g). About sterols in seafood from Gilbert Bay in Southern Labrador was reported by Copeman and Parrish (2004). They found that cholesterol was the major sterol in the molluscs investigated. Data about the cholesterol content of sharks and rays have been reported by Lytle and Lytle (1994), who found a variation in cholesterol content between 16 to 69 mg/100g. Commercial fish and crustacean species from the Arabian Gulf were assayed for cholesterol by Ewaidah (1993). The cholesterol content ranged from 65.2 mg/100 g to 146.2 mg/100 g. El. Sayed and others (1984) measured the cholesterol content in *Tilapia nilotica* and *Sparus auratus*. The cholesterol content varied between 50 and 170 mg/100g with the highest content in summer and the lowest in spring in *T. nilotica*. 10 selected marine fish in Malaysian waters were investigated by Osman and others (2001), for the cholesterol content. The total cholesterol content was generally low between 37 and 49 mg/100 g. Mathew and others (1999), found in 97 samples of 43 families differing cholesterol contents. The work, however, is only based on one specimen per species. DeKonig and others (1993) found in a number of South African fatty fish species an average cholesterol content of 130 mg/100 g.

Imre and Saglik (1998) found in nine common fish species purchased on the central fish market in Istanbul cholesterol contents between 43 mg/100 g and 75 mg/100 g. Two groups worked on the cholesterol content in sardines. Krzynowek and others (1992) determined in Maine sardines an average cholesterol content of 90 mg/100 g with little seasonal variation, while

De Leonardis and Macciola (2004) reported cholesterol contents of 93 mg/100 g (variation 67 to 131 mg/100 g) in Adriatic sardine fillets.

There are only few data on cholesterol in freshwater species. Bieniarz and others (2000) analysed the cholesterol content in some farmed freshwater fish species in Poland and found the lowest cholesterol content in European catfish (21 mg/100 g) while rainbow trout (muscle+skin) exhibited the highest content with 190 mg/100 g. 20 mg Cholesterol/100 g was detected by Lahti (1987) in vendace (*Coregonus albula*) from a Finish lake. Three Brazilian farmed freshwater species from the Platina basin were reported by Moreira and others (2001) to contain cholesterol contents of approx. 50 mg/100.

Concerning differences in the cholesterol content of wild and farmed fish species, Nettleton and Exler (1992) could demonstrate that there are no significant differences in the wild and farmed form of Channel catfish (*Ictalurus punctatus*) 58 mg/100 g and 61 mg/100 g, respectively, Coho salmon (*Oncorhynchus kisutch*) 48 mg/100 g and 51 mg/100 g, respectively and rainbow trout (*Oncorhynchus mykiss*) 60 mg/100 g and 59 mg/100 g, respectively.

The cholesterol content of red shrimp (*Aristeus antennatus*), pink shrimp (*Parapenaeus longirostris*) and Norway lobster (*Nephrops norvegicus*) caught off the South coast (Algarve) of Portugal was found to be 61 and 58 mg/100g for the shrimps and 60 mg/100 g for Norway lobster by Rosa and Nunes (2003). Cholesterol content in winter samples was higher than in summer samples. Cholesterol in several species of shrimp was assayed by Krzynowek and Panunzio (1989). The overall average was 152 mg/100g of edible portion of shrimp. Mohd.Omar and others (1995) published the cholesterol content of 5 Malaysian prawn species. The variation was not big among species ranging from 157 to 186 mg/100 g. The highest content was found in Rainbow prawn. Takada and others (1988) analysed 18 shrimp and prawn species for their cholesterol content and found that the total cholesterol was in the range of 90-150 mg/100 g in the edible portion of the shrimp. From 100 g shrimp tissue *(Penaeus aztecus*) isolated Johnston and others (1983) 201 mg/100 g cholesterol and 49 mg/100 g cholesterol esters. Also Uddin and others (2001) found high cholesterol values in crustacean shellfish from the Bay of Bengal varying between 130 mg/100 g in *Metapenaeus monocerus* to 161 mg/100 g in *Penaeus indicus*. The same authors also found very high contents in cephalopods (200 mg/100 g in *Loligo duvauceli* and 169 mg/100 g in *Sepia aculeate*. In Dungeness crab and in pink shrimp King and others (1990) found an average cholesterol content of 72 mg/100 g and 147 mg/100 g, respectively. In the same publication the authors analysed in molluscs: 48 mg/100 g in Pacific oyster, 37 mg/100 g in blue mussel, 36 mg/100 g in Manila clam and 231 (197-293) mg/100 g in California squid. Krzynowek and others (1989) investigated also 2 species of squid, *Loligo pealei* and *Illex illecebrosus*, and reported cholesterol content of 300 mg/100g (171 – 449 mg/100 g variation) and 212 mg/100g (108 – 336 mg/100 g variation), respectively. Extreme high cholesterol content in fish roe have been published firstly by Iwasaki and Harada (1984), who published cholesterol contents up to 655 mg/100 g in roe of Black Sea bream. The same authors investigated later (1985) also the roe of squid (*Dorytheutis bleekeri*) and crab (*Portunus trituberculatus*) which showed 374 mg/100 g and 494 mg/100 g, respectively.

Material and methods

Fish and shellfish

The fish used for the experiments were collected during 17 research cruises of the Fishery Research Vessels "Walther Herwig II" and "Walther Herwig III" in North Atlantic waters and "Clupea" in the Baltic Sea. Table 1 gives an overlook about cruises, areas and seasons. A compilation of the species investigated and their scientific names are given in Table 2.

The marine specimens were taken from hauls mostly made by bottom trawling. Life fish was killed by a blow on the head followed by gill cut and gutting. After bleeding the fish were cleaned in cold seawater and subsequently filleted and skinned. Whole fillets of both sides of the fish were chopped into small cubes of approx. 1 cm^3. To get a sub-sample representative of the whole fillet the cubes were mixed thoroughly and a sub-sample of ca. 50 g was taken. This was put in a polyethylene jar covered with a polyethylene lid and frozen in a blast freezer down to –39 °C. Until being analysed in the laboratory at land the samples were stored frozen at –25 °C. Roe was carefully removed from the opened belly and care was taken not to contaminate the roe with remains of liver or kidney.

At land samples were put in a freeze dryer and dried until water content was constant and < 0.5% of dry weight. The dried samples were finely milled in a ball mill and then used for cholesterol analysis.

Samples of cephalopods (octopus and squid) were obtained from German traders of frozen fish and brown shrimp from the Wadden Sea off Schleswig-Holstein (*Crangon crangon*) from a commercial shrimp processing establishment in North Germany. Samples of tropical and

Table 1. Cruises with fishery research vessels for sample collection for cholesterol analysis.

vessel	cruise no	year	month	area	no of samples
Walther Herwig II	110	1990	Sep/Oct	East/West Greenland	40
Walther Herwig II	115	1991	May/June	North Sea	27
Walther Herwig II	126	1992	July	North Sea	10
Walther Herwig II	137	1993	Sep/Oct	East/West Greenland	23
Walther Herwig III	150	1994	Aug	Barents Sea	64
Walther Herwig III	184	1997	May/June	Faroe Islands	79
Walther Herwig III	187	1997	Aug	Barents Sea	187
Walther Herwig III	194	1998	Mar	South Ireland/Biscay	42
Walther Herwig III	195	1998	Apr	Faroe Islands	232
Walther Herwig III	204	1999	Apr	Faroe/Shetland Islands	67
Walther Herwig III	210	1999	Sep	Skagerak/Kattegatt	79
Walther Herwig III	214	2000	Feb	North Sea	13
Walther Herwig III	217	2000	May/June	Barents Sea	185
Walther Herwig III	232	2001	Sep	Faroe/Shetland Islands	165
Walther Herwig III	243	2002	Sep	Westbritish/Ireland	69
Clupea	112	2001	Mar	Usedom/Baltic Sea	116
Clupea	143	2003	July	Usedom/Baltic Sea	103

Table 2. List of species investigated in this review.

Eel	*Anguilla anguilla*
Eelpout	*Zoaces viviparus*
Burbot	*Lota lota*
Blue Whiting	*Micromesistius poutassou*
Blue ling	*Molva dypterygia*
Bream	*Abramis brama*
Conger	*Conger conger*
American plaice	*Hippoglossoides platessoides*
Dogfish	*Squalus acanthias*
Megrim	*Lepidorhombus whiffiagonis*
Flounder	*Plathichthys flesus*
Perch	*Perca fluviatils*
Trout	*Salmo trutta*
Pout	*Trisopterus luscus*
Forkbeard	*Phycis blennoides*
Brown shrimp	*Crangon crangon*
Brill	*Scophthalmus rhombus*
Argentine	*Argentina sp.*
Grenadier	*Coryphaenoides rupestris*
Herring	*Clupea harengus*
John Dory	*Zeus faber*
Ocean quahaug	*Arcta islandica*
Scallop	*Pectinidae*
Cod	*Gadus morhua*
Norway lobster	*Nephrops norvegicus*
Cold water shrimp	*Pandalus borealis*
Wolffish	*Anarhichas spec.*
Pope, ruffe	*Gymnocephalus cernuus*
Dab	*Limanda limanda*
Gurnard	*Trigla spec.*
Salmon	*Salmo salar*
Ling	*Molva molva*
Lemon sole	*Microstomus kitt*
Capelin	*Mallotus villosus*
Cusk	*Brosme brosme*
Mackerel	*Scomber scombrus*
Blue mussel	*Mytilus edulis*
Mora moro	*Mora moro*
Octopus	*Octopus vulgaris*
Flying squid	*Todarodes sagittatus*
Polar cod	*Boreogadus saida*
Ocean perch	*Sebastes marinus, mentella*
Roach	*Rutilus rutilus*
Witch	*Glyptocephalus cynoglosus*
Sandeel	*Ammodytae*
Anchovy	*Engraulis encrasicolus*
Sardine	*Sardina Pilchardus*

Table 2. Continued.

Haddock	*Melanogrammus aeglefinus*
Houting	*Coregonus oxyrhynchus*
Plaice	*Pleuronectes platessa*
Greenland Halibut	*Rheinhardtius hippoglossoides*
Black Scabbardfish	*Aphanopus carbo*
Lumpsucker	*Cyclopterus lumpus*
Hake	*Merluccius merluccius*
Saithe	*Pollachius virens*
Angler	*Lophius piscatorius*
Sole	*Solea solea*
Sprat	*Sprattus sprattus*
Turbot	*Psetta maxima*
Pollack	*Pollachius pollachius*
Smelt	*Osmerus eperlanus*
Horse mackerel	*Trachurus trachurus*
Black Sea bream	*Mylio macrocephalus*
Edible crab	*Cancer pagurus*
White halibut	*Hippoglossus hippoglossus*
Whelk	*Buccinum undatum*
Whiting	*Merlangius merlangus*
Pikeperch	*Stizostedion Lucioperca*

subtropical shrimps and prawns were bought frozen at Hamburg fishmarket. These samples were treated in the same way as the marine fish samples prior to analysis.

All freshwater fish samples originated from catches off the coast of Mecklenburg-Vorpommern (Bodden) or were angled in Danish lakes South of Aarhus (Jutland) and have been treated as the marine fish samples.

In total approx. 1600 fish samples comprising 68 species have been analysed for cholesterol content. Additionally 75 shellfish samples, 171 commercial samples and 92 gonads (roe) have been analysed. The total number of analyses was approx. 4200.

Cholesterol determination by capillary gas chromatography

The method used is a modification of the method proposed by Naeemi and others (1995). The method was modified by Oehlenschläger (1999, 2000). Modified and newly added steps in the procedure are designated by (MOD).

200 – 250 mg (MOD) of freeze-dried, finely homogenised sample were weighed into a test tube. 25 µl of internal standard solution (1 mg 5-α-cholestane/ml cyclohexane) and 2.5 ml saturated methanolic potassium hydroxide solution (KOH) were added. The test tube was closed by a screw cap and shaken well by hand. Subsequently it was heated in a water bath at +80 °C for 30 min. It is advisable for complete saponification to shake the glass 1-2 times during tempering. The test tube was then cooled down under running tap water until temperature was

below 50 °C. Then 0.5 ml of magnesium chloride solution (25.41 g $MgCl_2$ * 6 H_2O/250 ml H_2O) (MOD) and 2.5 ml cyclohexane were added. The test tube was well shaken on a vortex mixer for 2 min and then centrifuged in a table centrifuge at maximum speed. The upper phase was completely transferred into another test tube filled with some anhydrous sodium sulphate (tip of a spatula) to remove residual water (MOD). Again the test tube was well shaken by hand. According to the cholesterol concentration in the sample the solution was diluted 1:10 (MOD) by cyclohexane prior to application or directly applied into a vial for the auto-sampler of the gas chromatograph.

Gas chromatograph: Hewlett Packard 5890 Series II plus with auto sampler HP 7673 and HP chemstation 3365; capillary column 30 m * 0.32 mm Ø with HP-1 or HP-5 (cross linked methyl siloxane); carrier and make-up gas helium; FID: synthetic air generated by Whatman zero air generator Model 75-83-220 and hydrogen generated by Packard hydrogen generator 9100; split inlet temperature 270 °C, FID temperature 300 °C, Helium flow 1.5 ml/min; temperature programme: 180 °C to 280 °C at 20 °C/min, then 10 min at 280 °C followed by 10 min at 300 °C (MOD).

The limit of detection was 0.5 µg cholesterol/g sample corresponding to 0.5 mg cholesterol/kg. The recovery rate was 95.7% with external standard and 97% with internal standard. This method can also measure other sterols but in this investigation cholesterol only was determined. Data are presented as median, 25% and 75% percentiles, respectively, and minimal and maximal values.

Result and discussion

Gadoid fish species

The cholesterol content of gadoid species (Table 3) was generally low. The species with the lowest (median) cholesterol content between 27 and 36 mg/100 g were: hake, blue ling, ling, pollack, grenadier, haddock and whiting. Blue whiting and cod were also low and only saithe and pout were higher than 40 mg/100 g. The variation was lowest in grenadier, ling, saithe and blue whiting, whereas blue ling, pout, cod, hake and Pollack differed more depending of catching area and season. These findings correspond well with those of Ackman and McLeod (1988) reporting the following cholesterol levels for gadoid fish species: cod (35 mg/100 g), cusk (32 mg/100 g), haddock (29 mg/100 g), hake (23 mg/100 g) and pollack (50 mg/100 g, but are lower than the cholesterol contents given by Candela and others (1997) with approx. 73 mg/100 g and Copeman and Parrish (2004) with 69 mg/100 g (using the Iatroscan technique) for cod.

Flat fish species

The cholesterol content of the flat fish species (Table 3) were in the same range like that of the gadoid species. Most flat fish species showed cholesterol contents between 30 and 40 mg/100 g only flounder, dab, Greenland halibut and White halibut were just above 40 mg/100 g. The variation was biggest in plaice, sole and White halibut – in these species the cholesterol content can be as low as 10 to 15 mg/100 g. Some specimen of Greenland halibut showed cholesterol values above 60 mg/100 g.

Table 3. Cholesterol contents (mg/100 g wet weight) in edible part of marine and freshwater fish, crustaceans and molluscs, median, 25% and 75% percentiles and min/max values.

Species	median	25% percentile	75% percentile	minimum	maximum
American plaice	38	35	42	22	50
Anchovy	30	19	30		
Angler	33	28	42	19	54
Argentine	56	47	62	32	99
Atlantic Salmon	26	24	27		
Black Scabbardfish	34	33	35	32	38
Blue ling	28	22	47	19	57
Blue mussel	18				
Blue Whiting	38	37	43	34	50
Bream	52	46	57	44	66
Capelin	73	71	77		
Cod	39	34	46	20	64
Cold water shrimp	107	193	126	81	119
Dab	43	40	47	39	50
Dogfish	42	37	47	29	69
Edible crab	29	30	31	23	42
Eel	51	31	70		
Eelpout	85	71	01	65	93
Flounder	42	40	46	30	60
Flying squid	140	132	153	75	227
Forkbeard	31	27	33	24	33
Greenland Halibut	42	36	45	31	68
Grenadier	35	33	37	26	44
Gurnard	38	32	40	28	52
Haddock	36	35	42	27	54
Hake	27	23	34	15	47
Herring	31	27	37	22	103
Horse mackerel	44	41	51	23	59
Houting	38	34	41	25	47
John Dory	52	48	57		
Lemon sole	33	24	39	17	45
Ling	31	28	34	21	44
Mackerel	33	24	43	14	55
Megrim	40	37	43	32	54
Mora moro	31	30	31		
Norway lobster	97	88	103	81	120
Ocean perch	41	35	47	20	53
Octopus	85	75	103	66	119
Perch	71	60	78	42	84
Pikeperch	63	53	72	42	79
Plaice	31	23	40	12	52
Pollack	31	25	37	23	39
Pope, ruffe	88	84	94	83	99
Pout	47	40	41	20	57

Table 3. Continued.

Species	median	25% percentile	75% percentile	minimum	maximum
Roach	44	40	48	30	57
Saithe	45	40	50	27	69
Sandeel	37	36	38	32	44
Sardine	24	23	25	18	27
Smelt	70	64	81	54	109
Sole	33	28	38	8	47
Sprat	40	35	43	25	52
Trout	27	22	30	17	32
Turbot	39	32	47		
White halibut	45	41	51	14	62
Whiting	36	32	44	23	50
Witch	36	33	37	31	48
Wolffish	43	38	48	15	68

Other marine fish species

The fish species with the lowest cholesterol content was identified as Atlantic salmon with 26 mg/100 g (Table 3). Extremely low were *Mora moro*, angler, black scabbardfish. Most species were in the range between 30 and 40 mg/100g, but some species had significant higher cholesterol contents like argentine (56 mg/100 g), smelt (70 mg/100 g) and capelin (73 mg/100 g). Both latter species belong to the family Osmeridae and it might be a characteristic of this family to contain more cholesterol in their edible parts than others. There is also evidence that the cholesterol content in fish from tropical areas is higher than in fish from temperate climate areas. Ewaidah (1993) assayed 11 species from the Arabian Gulf and found cholesterol contents between 54 mg/100 g and 94 mg/100 g with an average of 65 mg/100 g. This is clearly above the average for fish species from the Northeast Atlantic which is approx. 40 mg/100 g.

In this investigation the cholesterol content of sharks and rays was not included. However, Lytle and Lytle (1994) have analysed four shark and ray species for their cholesterol content. Cholesterol contents in Southern Stingray (*Menticirrhus americanus*) ranged from 57 to 69 mg/100 g and in Atlantic stingray (*Dasyatis Sabina*) from 30 to 39 mg/100 g. In blacktip shark (*Carcharhinus limbatus*) the range was between 34 and 58 mg/100 g and in Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) between 16 and 23 mg/100 g. This shows that the cholesterol contents in sharks and rays (cartilaginous fish species) is of the some order as in bony fish species.

Small fatty pelagic fish

The pelagic species herring, mackerel, anchovy and sardine were all very low in cholesterol (24 to 33 mg/100 g) despite of the fact that they are fatty fish species (Table 3). The low cholesterol content found in sardine is in contrast to the high values (93 mg/100 g average) reported for this species from the Adriatic coast by other authors (DeLeonardis A and Macciola V, 2004). Sprat and horse mackerel were somewhat higher but still in the low range. The variation in all species was low with the exception of herring. The cholesterol content of herring can

vary from 20 mg/100 g to just above 100 mg/100 g as a function of catching area, season, state of maturity and other factors. However, the majority of specimen analysed do not vary too much.

Freshwater fish species

The cholesterol content of the edible part of freshwater species (Table 3) was found to be generally higher than the cholesterol content of marine fish species. Trout and houting are exceptions, the other species exhibit cholesterol contents above 50 mg/100 g. High contents were especially found in eelpout, pope or ruffe, which is not a commercially used food fish, perch and pikeperch. Most of the freshwater species are lean fish species comparable in fat content to gadoids. The higher cholesterol content in freshwater fish seems to be characteristic for them. This is supported by cholesterol levels found in perch (92 mg/100 g), pike (*Esox lucius*) (63 mg/100g), pikeperch (79 mg/100 g), vendace (74 mg/100 g) and whitefish (*Coregonus lavaretus*) (49 mg/100 g) by Piironen and others (2002).

Roe (gonads) of fish species

In Figure 1 are shown the cholesterol contents in roe (fish eggs) of all species investigated. At a first glance it can be seen that the cholesterol contents of the roe was 5 to 10 times higher than the content in the edible part of fish (muscle, fillet). Most fish roe scattered around 200 mg/100g, but some species like hake and plaice were much higher. The reason for the extremely high cholesterol content in roe is the fact that in the single egg enough cholesterol has to be present to form the lipid bi-layers in the cells during nuclear division of the developing fish embryo. Consequently, all fishery products processed on the basis of roe (caviar, smoked or canned cod roe, caviar substitute, keta-caviar, trout-caviar, caviar spread *etc.*) always contain high amounts of cholesterol. Very high cholesterol contents in the roe of rainbow trout and vendace have been determined by Piironen and others (2002) with 409 mg/100 g and 743 mg/100 g, respectively.

Shellfish

The mussels investigated, scallop, blue mussel and ocean quahaug were with content around or lower 30 mg/100 g very low in cholesterol. The crustaceans, Norway lobster and cold water shrimp were all high in cholesterol (Table 3). The welk lies in the same area (120 mg/ 100 g). An exception forms the edible crab (*Cancer pagurus*), which is the only crustacean in this investigation with a very low cholesterol content (29 mg/100 g). The two cephalopods measured, flying squid and common octopus exhibit high cholesterol contents, 144 and 84.5 mg/100 g, respectively (Table 3).

Crustaceans and molluscs contain a number of other sterol as cholest-5,22-dien-3-ß-ol and 24-methylcholesta-5,22-dien-3-ß-ol, but the main sterol component is always cholesterol. Piretti and Serrazanetti (1980) could show that the prawn *Penaeus cheraturus* contained 94.5% (173.6 mg/100 g) of its total sterols as cholesterol and Sica (1980) demonstrated that 91.1% of all sterols in *Loligo vulgaris* were cholesterol. In other crustacean species like in *Squilla mantis* from the Mediterranean Sea (Serrazanetti and others 1990) the cholesterol content can be a much lower fraction of the total sterol content (55.7%).

Cholesterol and fat content

Figure 2 is a plot of the cholesterol contents of all fish specimen investigated versus their respective dry matter. Since dry matter in fish increases strictly parallel with fat content this

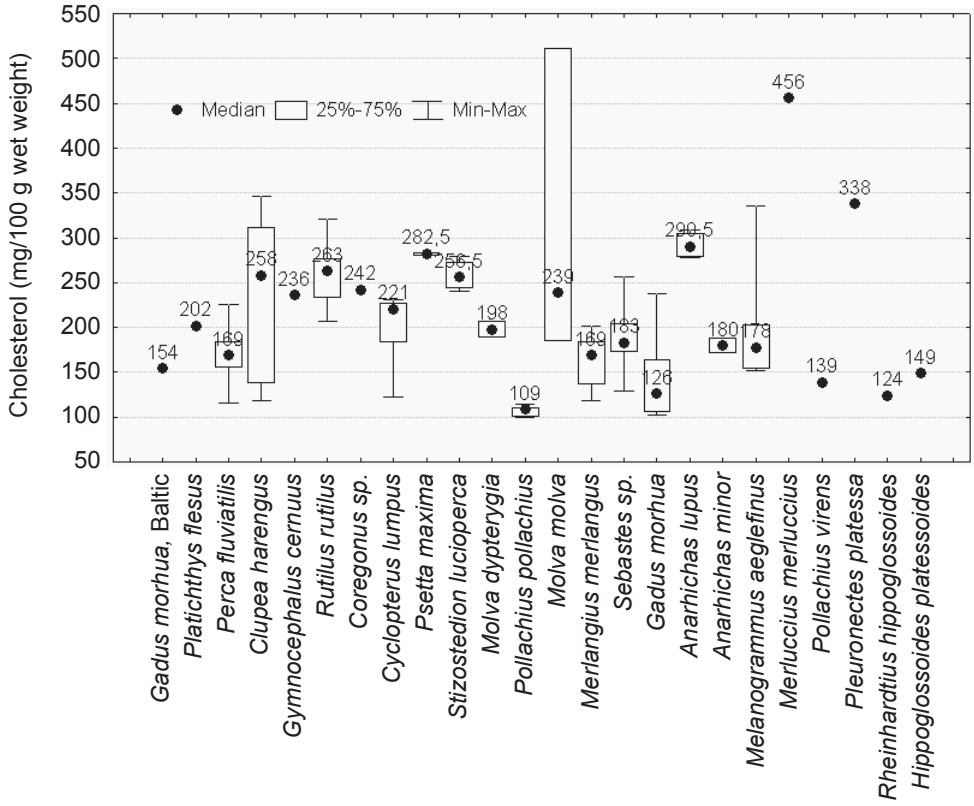


Figure 1. Cholesterol content in gonads (roe) of some fish species investigated, 25% and 75% percentiles and min/max values.

figure can be used for statements about cholesterol and fat content. In the figure there are three areas. Area 1 to the lower left is an area formed by specimen with a low cholesterol content and a low to medium fat content. The second area to the lower right, is formed by the specimen with high fat content and low cholesterol content and the third area, to the upper left is formed by species with a high cholesterol content but a low fat content. Area 3 is formed mainly by freshwater samples, area 2 by fatty pelagic species and area 1 by the marine fish samples. It is obvious that there are no samples in this investigation, which are found in the area "high cholesterol, high fat content", since this is not existing. It must be stressed that almost all fatty fish samples are low in cholesterol and that somewhat higher cholesterol contents are never found in fatty fish, but in lean freshwater fish species.

Figure 3 supports these findings. The plot of cholesterol contents in all mackerel measured versus dry matter content (s.a.) shows that the cholesterol content decreases the fattier the fish is. This can be explained by the fact that the cholesterol is a component of the cell membranes of the fish tissue. The number of cells is fixed and only subject to minor variations. Since there is no accumulation of cholesterol in depot fat, subcutaneous fat layers *etc.* the cholesterol content is almost constant and if the fish accumulates fat the cholesterol content measured goes down.

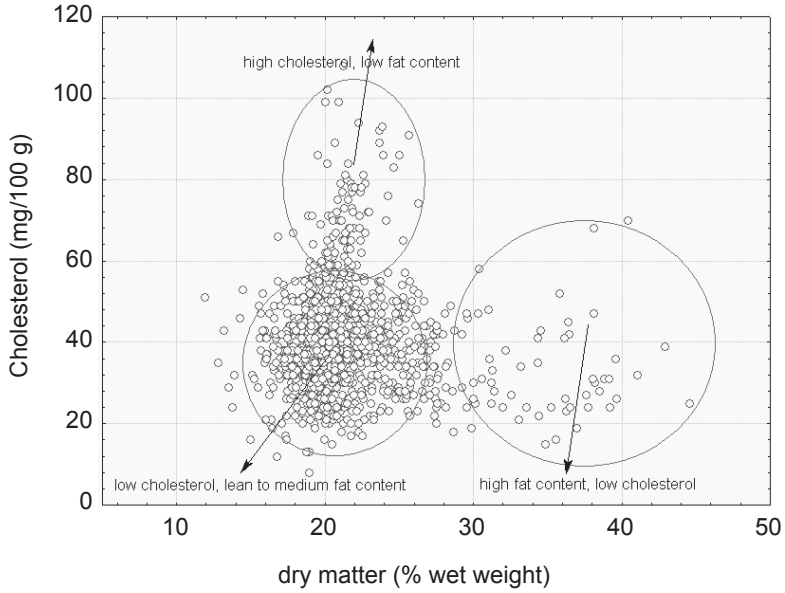


Figure 2. Plot of cholesterol content and dry matter (fat) in edible part of all fish specimen investigated.

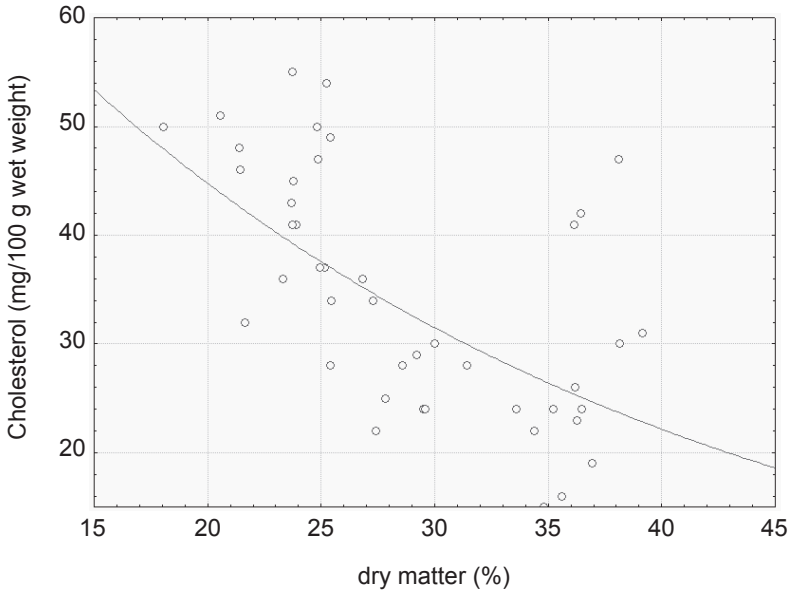


Figure 3. Plot of cholesterol content and dry matter of mackerel specimen with different fat contents.

The situation, however, is totally different in fish roe (Figure 4). The plot shows that the fattier the roe is (dry matter) the more the content of cholesterol. Here cholesterol has another function. It is accumulated in a depot to be present when the cell division takes place to form a part of the cell membrane. The fattier the fish roe, the more cholesterol it contains.

Commercial samples

The results of the commercial samples (Table 4) support the findings in the collected samples onboard the vessels. Fish roe products (Russian caviar, caviar spread, canned cod roe) are all high in cholesterol with Russian caviar being on top. Commercial quick frozen octopus is also high in cholesterol exceeding the content of the octopus sampled onboard. North Sea shrimps are highest in cholesterol of all crustaceans. Smoked salmon produced from Norwegian farmed salmon is very low with 26 mg/100g. This corresponds well with the value for raw salmon in Table 3 (20 mg/100 g) and can be explained by the water loss during processing of the smoked product.

Imported tropical and subtropical shrimps gave almost identical contents in two independent trials (140 and 150 mg/kg). High cholesterol contents in tropical and subtropical shrimps and prawns have been reported by other authors: Krzynowek and Panunzio (1989) found in Northern shrimp (*Pandalus borealis*) 135 - 186 mg/100 g, in Georgia white shrimp (*Peneaus setiferous*) 139 - 147 mg/100 g, in Honduras pink shrimp (*Penaeus durarum notialis*) and in Ecuador white shrimp (*Penaeus vannamei*) 150 mg/100 g, in Texas brown shrimp (*P. aztecus*) 151 mg/100 g. Also Gopakumar and Nair (1975) reported for *Metapenaeus monocerus* from brackish water 90 mg/100 g and for the marine species *M. dobsoni* 170 mg/100 g, *M. affinis* 133 mg/100 g, *P. indicus* 118 mg/100 g and *Parapenaeopsi stylifera* 130 mg/100 g. This is supported by Mohd.

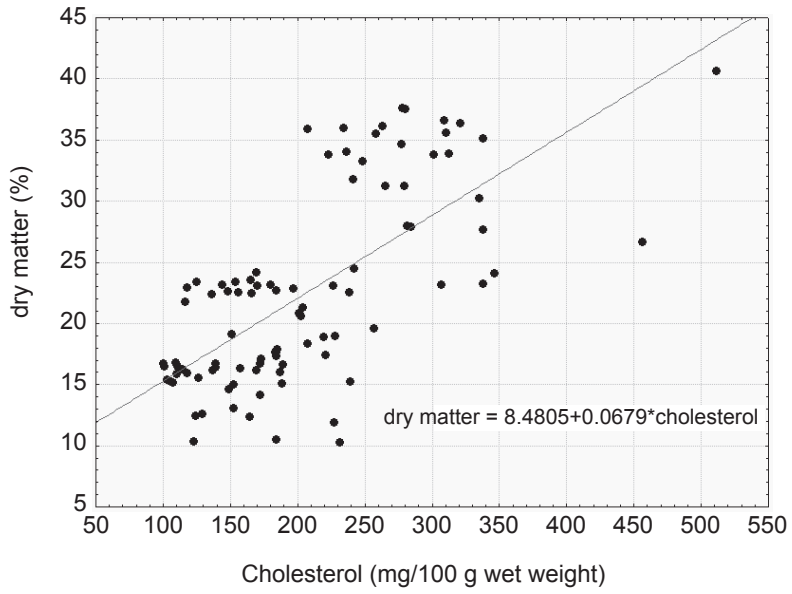


Figure 4. Plot of cholesterol content and dry matter of gonads (roe) of all species analysed.

Table 4. Cholesterol content (mg cholesterol/100 g wet weight) in commercial samples of fishery products, arithmetic mean and standard deviation.

Fishery product	Arithmetic mean	Standard deviation
Caviar, Russia, Malossol	273	29
Caviar spread, Scandinavia, different brands	97	83
Cod roe, canned, Denmark	154	
Octopus, deep frozen	114	5
Deep frozen tropical and subtropical shrimps	141	19
North Sea shrimps	194	9
Deep frozen shrimps, imported, 1997	151	16
Smoked salmon fillets, Norway	27	4

Omar and others (1995) who also found significant amounts in Malaysian prawn species: *P. monodon* 178 mg/100 g, *P. merguiensis* 157 mg/100 g, *Metapenaeus brevicornis* 160 mg/100 g, *Parapenaeopsis hardwickii* 168 mg/100 g and *Parapenaeopsis sculptilis* 186 mg/100 g. *P. aztecus* was also investigated by Johnston and others (1983) and exhibited 200 mg/100 g of free cholesterol. Uddin and others (2001) confirmed these findings by analysing shrimps and prawns from the Bay of Bengal: *P. indicus* 161 mg/100 g, *P. monodon* 132 mg/100 g and *Metapenaeus monocerus* 130 mg/100 g. In this paper also the extremely high cholesterol contents in cephalopods were confirmed in two species: squid (*Loligo duvauceli*) 200 mg/100 g and cuttle fish (*Sepia aculeate*) 169 mg/100 g.

Seasonal and area variation

Cod was caught in 5 different areas from March to October in different years. From Figure 5 it seems that the lowest cholesterol content was found in cod caught in Greenland waters or Faroese/Shetland waters. The highest content was found in the Barents-Sea. A closer look, however to the large variations and the min/max values – with the exception of the cod caught in coastal Baltic Sea – shows that there is no great variation in cholesterol content as a factor of season and catching ground in cod. In Figure 6 a frequency distribution plot of cholesterol content in approx. 200 specimen of cod from different catching grounds and seasons is shown proving that the cholesterol content in edible part of cod is normally distributed if a sufficient large number of specimen is analysed.

As shown the cholesterol content in marine fish is varying considerably inter and intra species, therefore it is not possible to use the cholesterol content in edible part of fish for the determination of the fish species as reported earlier and proposed by Wurziger and Hensel (1967).

Conclusion

From the results presented it can be concluded that:

- fish muscle is low in cholesterol;
- fatty fish is very often low in cholesterol;
- freshwater fish is higher in cholesterol compared with marine fish;
- shrimps and prawns, squid and octopus are high in cholesterol;

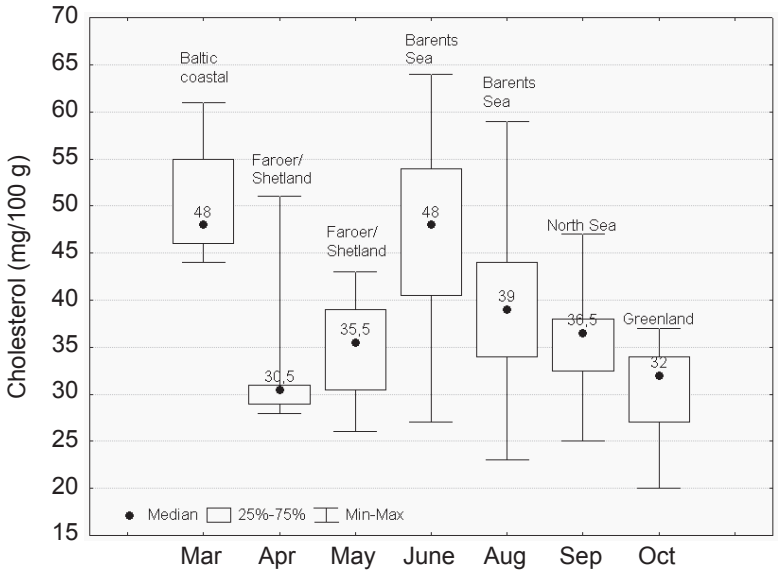


*Figure 5. Cholesterol content in edible part of cod (*Gadus morhua*) caught in different catching areas in different months.*

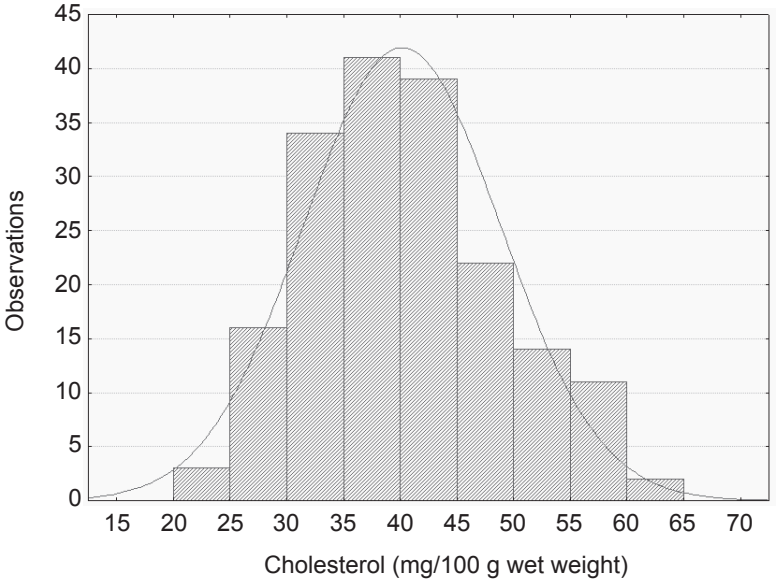


*Figure 6. Frequency distribution of cholesterol content (mg/100 g on a wet weight basis) in edible part of cod (*Gadus morhua*) from different catching areas, seasons and years.*

- mussels and edible crab are low in cholesterol;
- roe (gonads) are always very high in cholesterol;
- no significant correlations was found with season and catching ground;
- there is an intrinsic variation between specimen of a given species.

In commercial samples the cholesterol content of smoked salmon is low, in imported tropical and subtropical shrimps and in local shrimps always high, in canned roe products and caviar products always high and Russian caviar has the highest cholesterol content.

Acknowledgements

The author wants to thank the crews and the masters of the vessels for their assistance and help and my co-workers for their enthusiasm in the work and their continuous efforts to create this huge data base. Special thanks to Hans-Jürgen Knaack for sampling onboard and to Tanja Pieplow for all the gas chromatographic analyses.

References

Ackman RG, McLeod C. 1988. Total lipids and nutritionally important fatty acids of some Nova Scotia fish and shellfish food products. Can Inst Food Sci technol J 21:390-398.

Anon 1999. Nutrient value of some common foods. Canadian Government Publishing, Ottawa, 54 p.

Bieniarz K, Koldras M, Kaminski K, Mejza T. 2000. Fatty acids and cholesterol in some freshwater fish species in Poland. Folia Univ Agric Stetin. 214(27):21-44.

Candela M, Astiasaran I, Bello J. 1997. Effects of frying and warmholding on fatty acids and cholesterol of sole (*Solea solea*), codfish (*Gadus morhua*) and hake (*Merluccius merluccius*). Food Chem 58:227-231.

Copeman LA, Parrish CC. 2004. Lipids classes, fatty acids, and sterols in seafood from Gilbert Bay, Southern Labrador. J Agric Food Chem 52:4872-4881.

DeKoning AJ, Hearshaw KD, van der Merwe G. 1993. Free and esterified cholesterol in a number of South African fish oils and their corresponding meals. Fat Sci Technol 95:27-31.

De Leonardis A, Macciola V. 2004. A study on the lipid fraction of Adriatic sardine filets (*Sardina pilchardus*). Nahrung/ Food 48:209-212.

El-Sayed MM, Ezzat AA, Kandeel KM, Shaban FA. 1984. Biochemical studies on the lipid content of *Tilapia nilotica* and *Sparus auratus*. Comp Biochem Physiol 79B:589-594.

Ewaidah EH. 1993. Cholesterol, fat and food energy content of selected raw and cooked commercial fish species from the Arabian Gulf. Ecol Food Nutr 30:283-292.

Feeley RM, Criner PE, Watt BK. 1972. Cholesterol content of foods. J Amer Diet Ass 61:134-149.

Gopakumar K and Rajendranathan Nair M. 1975. Lipid composition of five species of Indian prawns. J Sci Food Agric 26:319-325.

Idler DR, Wiseman P. 1971. Sterols of crustacea. Int J Biochem 2:91-98.

Imre S, Saglik S. 1998. Fatty acid composition and cholesterol content of some Turkish fish species. Turk J Chem 22:321-324.

Iwasaki M, Harada R. 1984. Cholesterol content of fish gonads and livers. Bull Jap Soc Sci Fish 50:1623.

Iwasaki M, Harada R. 1985. Proximate and amino acid composition of the roe and muscle of selected marine species. J Food Sci 50:1585-1587.

Johnston JJ, Ghanbari HA, Wheeler WB, Kirk JR. 1983. Characterization of shrimp lipids. J Food Sci 48:33-35.

Kanazawa A. 2001. Sterols in marine invertebrates. Fisheries Sci 67:997-1007.

King I, Childs MT, Dorsett C, Ostrander JG, Monsen ER. 1990. Shellfish: Proximate composition, minerals, fatty acids, and sterols. J Am Diet Ass 90:677-685.

Kritchevsky D, Tepper SA, DiTullo NW, Holmes WL. 1967. The sterols of seafood. J Food Sci 32:64-66.

Krzynowek J. 1985. Sterols and fatty acids in seafood. Food Technol 39:61-68.

Krzynowek J, D'Entrement DL, Murphy J. 1989. Proximate composition and fatty acid and cholesterol content of squid, *Loligo pealei* and *Illex illecebrosus*. J Food Sci 54:45-48.

Krzynowek J,, Panunzio LJ. 1989. Cholesterol and fatty acids in several species of shrimp. J Food Sci 54:237-239.

Krzynowek J, Uljua DS, Panunzio LJ, Maney RS. 1992. Factors affecting fat, cholesterol, and Omega-3 fatty acids in Maine sardines. J Food Sci 57:63-65+111.

Lahti E. 1987. Total lipid and cholesterol contents of liver and muscle in some fish species, especially vendace (*Coregonus albula* L.) in Finland. Arch Hydrobiol. 110:133-142.

Lytle JS, Lytle TF. 1994. Fatty acid and cholesterol content of sharks and rays. J Food Comp Anal 7:110-118.

Mathew S, Ammu K, Viswanathan Nair PG, Devadasan K. 1999. Cholesterol content of Indian fish and shellfish. Food Chem 66:455-461.

Mohd. Omar AK, Abd Malik O, Ismail N. 1995. Cholesterol content of some Malaysian marine prawn species. ASEAN Food J 10:39-40.

Moreira AB, Visentainer JV, de Souza NE, Matsushita M. 2001. Fatty acids profile and cholesterol contents of three Brazilian Brycon freshwater fishes. J Food Comp Analysis 14:565-574.

Naeemi ED, Ahmad N, Al-sharrah TKM, Behbahani M. 1995. Rapid and simple method for determination of cholesterol in processed food. JAOAC Int. 78:1522-1525

Nettleton JA, Exler J. 1992. Nutrients in wild and farmed fish and shellfish. J Food Sci 57:257-260.

Oehlenschläger J. 2000. Cholesterol content in edible part of marine fatty pelagic fish species and other seafood. In: Georgakis SA, editor, Proceedings 29th WEFTA Meeting 1999,. Thessaloniki: Greek Society of Food Hygienists and Technologists. p 107-115

Oehlenschläger J. 1999. Gaschromatographische Bestimmung des Gesamtcholesterolgehaltes im verzehrbaren Anteil (Filet) von Meeresfischen und Garnelen. Lebensmittelchemie 53:86.

Oehlenschläger J. 1998. Cholesterol in Krebstieren – Analytik und Gehalte, Angewandte Wissenschaft, Heft 469:75-81.

Osmann H, Suriah AR, Law EC. 2001. Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. Food Chem 73:55-60.

Piironen V, Toivo J, Lampi A-M. 2002. New data for cholesterol contents in meat, fish, milk, eggs and their products consumed in Finland. J Food Comp Analysis 15:705-713.

Piretti MV, Serrazanetti GP. 1980. Investigation of the sterol constituents of the prawn *Penaeus cheraturus*. J Amer Diet Ass 61:134-149.

Rosa R, Nunes ML. 2003. Nutritional quality of red shrimp, *Aristeus antennatus* (Risso), pink shrimp, *Parapenaeus longirostris* (Lucas) and Norway lobster, *Nephrops norvegicus* (Linnaeus). J Sci Food Agric 84:89-94.

Serrazanetti GP, Conte LS, Baraldi R. 1990. Sterol content in muscular tissue of *Squilla mantis*. Comp Biochem Physiol 96B:811-814.

Sica D. 1980. Sterols from some molluscs. Comp Biochem Physiol 65B:407-410.

Souci SW, Fachmann W, Kraut H. 2000. Food Composition and Nutrition Tables. CRC Press, New York, 1182 p.

Takada K, Aoki T, Kunisaki N. 1988. Proximate composition, free amino acid, fatty acid, mineral and cholesterol content in imported frozen shrimps. Nippon Suisan Gakkaishi 54:2137-2179.

Teshima S-I 1991. Sterols of crustaceans, molluscs and fish. In: Patterson GW, Nes WD, editors, Physiology and biochemistry of sterols. Champaign: Amer Oil Chem Soc. p 229-256

Uddin M, Jahan P, Ahmad MU. 2001. Fat and cholesterol content in some fish and shellfish of Bay of Bengal. Asian J Chem 13:1227-1230.

Wurziger J, Hensel G. 1967. Über den Cholesterin-Gehalt in Fischfilets zur Ermittlung der Fischart. Fette Seifen Anstrichm 69:937-942.

Capelin oil for human consumption

Margrét Bragadóttir, Ása Þorkelsdóttir, Irek Klonowski and Helga Gunnlaugsdóttir
Icelandic Fisheries Laboratories, Skulagata 4, 101 Reykjavik, Iceland

Abstract

Oil-in-water emulsion with capelin oil was made as egg-free mayonnaise containing whey protein emulsifier. Mayonnaise was enriched with 20% fish oil, with and without antioxidant (tocopherol mixture (200 mg/kg)), in addition to a control sample without fish oil. The quality and stability at 10 °C was evaluated by conjugated dienes/ trienes and sensory analysis, besides stability tests at 60 °C. Lipid oxidation of the mayonnaise was not increased by fish oil addition, but the fish oil flavour was apparent. Tocopherol was ineffective as antioxidant in the fish oil enriched mayonnaise and tended to increase oxidative degradation. Processing optimisation is necessary in order to conclude if capelin oil is suitable in functional foods with sensory acceptance, but this study indicated that adequate stability could be obtained.

Keywords: fish oil, mayonnaise, lipid oxidation, sensory evaluation

Introduction

The significance of fish oils as important dietary source of valuable fatty acids of long chained omega-3 type has been confirmed by research. The diverse beneficial health effects of a diet high in long chain n-3 polyunsaturated fatty acids has been demonstrated by epidemiological studies and reviewed extensively (Uauy and Valenzuela 2000; Simopoulos and Cleland 2003; Schmidt and others 2004). In order to increase the consumption of marine fatty acids several attempts have been done to incorporate fish oil into different foods (Young 1990; Kolanowski and others 1999; Medina and others 2003). Incorporation of omega-3 fatty acids and fish oils into functional food is limited by their high susceptibility to oxidative degradation. Oil-in-water emulsions may be an effective method to deliver omega-3 fatty acids into foods, rather than bulk lipids, because emulsions are more frequently found in actual food products (Coupland and McClements 1996; McClements and Decker 2000). An emulsion consists of thee regions: the interior of a droplet, the continuous phase and the interfacial region, where systems consisting of oil droplets dispersed in an aqueous phase are known as an oil-in water emulsion (Coupland and McClements 1996). Emulsifiers are situated at the oil-water interface because they contain both hydrophilic and hydrophobic groups, and their function is to prevent emulsions from separating into oil and water phases. Mayonnaise is an example of oil-in-water emulsion that is traditionally made of egg yolk as the emulsifying agent. Other ingredients in mayonnaise are oil, water, vinegar, salt, sugar and mustard. Potassium sorbate and sodium benzoate are often added to mayonnaise to inhibit microbial growth. Vinegar, salt, sugar and mustard are added to mayonnaise as flavouring ingredients, but all of these ingredients also seem to play an important role for the physical stability of emulsions (McClements and Decker 2000). Besides egg yolk, many emulsifying agents have been applied in oil-in-water emulsions. Whey proteins have gained much attention as they have been found to increase oxidative stability of emulsions, including those containing omega-3 fatty acids (Hu and others 2003, Djordjevic and others 2004b). Whey proteins are believed to inhibit oxidation by chelating

prooxidant transition metal ions, inactivate free radicals or by forming physical barriers between water-soluble prooxidants and lipids at the lipid-water interface (Donnelly and others 1998; McClements and Decker 2000).

The fish oil consumption in Iceland has to date almost entirely been in the form of a daily spoon of cod liver oil as a health remedy. Other sources of fish oils derive from fish meal production on small, whole fish, such as capelin (*Mallotus villosus*), which is the major part of the Icelandic fish oil production. To date little efforts have been made in order to exploit the capelin oil for human consumption. The capelin oil produced in Iceland is mainly exported as an ingredient for use in aquaculture and animal feeds, and the value of this feed grade fish oil is less than half the price of fish oil for human consumption (*e.g.* cod-liver oil). Consequently, the possibilities to increase the value of capelin oil by incorporation it into food products are encouraging. In order to achieve food grade quality capelin oil it is necessary to take into account the natural variation in the capelin stock as raw material and it might also require optimisation of handling and processing techniques. The oil content in capelin can vary from 2–20% depending on season, and the quality of the crude oil can vary as well, as reflected in the content of free fatty acids, the content of natural antioxidants like tocopherol and astaxanthin, as well as in fatty acid profile (Bragadóttir and others 2002). The fatty acid profile of capelin is unusual, with extraordinary high concentration of long chained monounsaturated fatty acids (MUFA´s), ranging from 46-57% and omega-3 fatty acids (C20:5 + C22:5 + C22:6) ranging from 12.5 to 18% of total fatty acids (Bragadóttir and others 2002).

The present investigation was undertaken to evaluate the possibilities to produce food products containing capelin oil with emphasis on the best suitable means to prevent lipid oxidation. Capelin oil was included in egg-free mayonnaise, containing commercial emulsifier mixture with milk proteins. Mayonnaise made with milk proteins was selected as a food product for capelin oil incorporation, as oil-in-water emulsions made with milk proteins have been extensively studied for the past years, and found to be promising to prevent lipid oxidation and deliver omega-3 fatty acids into foods.

Materials and methods

Materials

The crude fish oil from capelin (*Mallotus villosus*) was provided by Síldarvinnslan hf., fish meal factory in Siglufjördur, during winter season. The fish oil was distilled in a bench top molecular distiller, type: KDL (UIC GmbH, Alzenau-Hörstein, Germany) at 185 ± 2 °C. The oil was packed under nitrogen gas into 1 L brown flasks and kept at -24 °C until used. The composition of fatty acids was; saturated fatty acids: 16.8%, monounsaturated fatty acids: 73.3%, polyunsaturated fatty acids: 9.9%, and thereof n-3 fatty acids: 5.9%. Soybean oil was obtained from Kjarnavörur hf, Garðabær, Iceland, the importer of Victoria-refined and deodorized soya oil (produced in Holland by Vereenigde Oil Fabrieken). The composition of fatty acids was; saturated fatty acids: 14.2%, monounsaturated fatty acids: 25.2%, polyunsaturated fatty acids: 60.7%, and thereof n-3 fatty acids: 6.7%. Coviox T-70, natural 70% tocopherol mixture was purchased from Cognis GmbH (Düsseldorf, Germany). The vinegar, mustard, salt (NaCl) and sugar were purchased from a local supermarket. Sodium benzoate was purchased from Merck (Darmstadt, Germany). The Grindsted™ FF1110 stabiliser system was provided by Danisco A/S (Langebrogade, Copenhagen), containing; milk protein (whey protein isolate), acetylated distarch adipate (E1422), guar gum (E412) and sodium alginate (E401).

Preparation of mayonnaise

The mayonnaise was made by a recipe from Danisco, Culinary Manual (Langebrogade, Copenhagen), on 70% egg free mayonnaise, by a cold batch process in a mixer (Braun Electronic, type 4265, Germany). Each batch contained by weight; distilled water (18.15%), salt (1.00%), sugar (2.00%) and sodium benzoate (0.10%) that were mixed together. Grindsted™ FF1110 (1.40%) was pre-mixed with oil in a ratio of 1:2 and added to the mixture. The oil (70.00%) was continuously emulsified into the water phase for 25 min. Finally, 10% vinegar (3.50%) and mustard (1.50%) were blended together and added to the emulsion. Three samples were prepared; one with soya oil and two with soya oil mixed with fish oil, with and without addition of tocopherol as antioxidant (Table 1). For shelf life testing, the mayonnaise samples were vacuum packed into glass jars (100 mL) for storage in the dark at 10-12 °C, to induce oxidation and resemble inadequate refrigeration during storage and in the chill chain of the product.

Free fatty acids

Free fatty acids (FFA) were determined in the oils that were solubilized in alcohol/diethyl ether (1:1) and titrated with diluted NaOH (AOCS 1998).

Peroxide value

Peroxide value of oils was measured by iodometric titration according to AOAC official method 965.33 (AOAC 1990).

Anisidine value

Anisidine value of oils was determined by the reaction of aldehydic compounds in oil and *p*-anisidine, and absorbance measured at 350 nm, according to standard methods (IUPAC 1987).

Conjugated dienes (CD) and conjugated trienes (CT)

The determination of the absorbance in the UV spectrum of the samples was measured at 232 nm as conjugated dienes and at 268 nm as conjugated trienes according to standard methods (IUPAC 1987), with minor modifications. Mayonnaise emulsions (0.150 g) were dissolved in methanol (20-25 mL) and mixed for 20 seconds with Ultra-Turrax homogenizer (type T25, IKA Werke, Staufen, Germany). The samples were centrifuged at 6500*g* for 5 min and the absorbance of the supernants measured. Two measurements were performed on duplicate samples and the results expressed according to the following formula: CD or CT = A_{232} or A_{268} / c x d; where A is the absorbance reading at 232 or 268 nm, c denotes the concentration of the solution in g per 100 mL and d is the length of the cell, in cm.

Table 1. Combinations of oils for the mayonnaise samples.

Code name	Soya oil (%)	Fish oil (%)	Tocopherol (mg/kg oil)
S	100	-	-
F	80	20	-
T	80	20	200

pH
The pH was measured using a puncture, combination electrode (SE 104, Mettler Toledo, Greifensee, Switzerland) connected to a pH meter (Knick-Portamess 913 pH, Berlin, Germany).

Sensory analysis
Samples were evaluated by Quantitative Descriptive Analysis (QDA) method (Stone and Sidel 1985). The method assumes detailed description of a product, such as odour, flavour, appearance and texture. List of attributes are defined and used with unstructured scale. The Icelandic Fisheries Laboratories sensory panel was trained in two sessions. Members had several years of experience in evaluating rancidity of fish, fish oils and vegetable oils and have been trained according to international standards (ISO 1993). Freshly made mayonnaise with pure soya oil, soya and fish oil (70:30) as well as soya and rancid fish oil (70:30) was used for training the panel. The panel compiled 16 descriptive attributes for mayonnaise, ranging from not present to strong; each for both odour and flavour: acetic acid, oily, musty, painty, fish oil, rancid, acidic, and for texture and appearance; creamy, clammy and colour (white/yellow).

Sensory assessments were carried out by seven to eight assessors (age range 30 - 60). Six samples were evaluated (three different samples each in duplicate) in two sessions, three at each. Sensory analysis, data collection and data analysis were done in the sensory program Fizz version 1.3 (Biosystemes, France). The order of presentation of samples to the panelists was balanced to minimize possible carry-over effects between samples. All observations of samples were conducted under standardized conditions, with as little interruption as possible, at room temperature, and under white fluorescent light. The mayonnaise was presented to the panelists in small, transparent, disposable plastic cups covered with an aluminium foil, along with water and crackers for oral rinsing between samples.

Oxidative stability
The oxidative stability of mayonnaise was measured electronically under oxygen pressure (5 bars) in an Oxipres apparatus (Mikrolab Aarhus A/S, Højbjerg, Denmark). Samples (7 g) were weighed into reaction flasks (125 mL) and the pressure signal was recorded at 60 °C. Each sample was measured in duplicate and the results presented as mean values.

Data analysis
Statistical analysis was done on the data by analysis of variance (ANOVA) on Number Cruncher Statistical Software (NCSS 2000 and Pass Trial, Kaysville, Utah). Duncan comparison test used to determine differences between samples ($P < 0.05$).

Results and discussion

Measurements on oils as raw materials in mayonnaise
Efforts were made to ensure that the oils used as raw materials for the mayonnaise were of high quality. The peroxide value (PV) of the soya oil was 1.0 meq/kg, whereas the PV of the distilled fish oil was not detected. The anisidine value (AV) of the fish oil was therefore also measured to verify its quality, and was found to be low, with AV of 2.5. The range of AV in commercial capelin oil (58 samples) measured at our lab were approximately 2 to 17, with an average of roughly 6. These samples were mostly made up of crude capelin oil that usually has lower AV than more processed oil. Thus, the AV of crude capelin oil was found to increase from 7.4 to 8.5 in alkali-refined oil, increasing further to 16.2 after bleaching of the oil, and

the AV ended in 21.2 after deodorization (Bragadóttir and others 1992). The fish oil in this study was therefore only refined by molecular distillation in order to minimize the negative effects of advanced processing on its stability. Oil purification by molecular distillation has been described as an effective method for deodorizing and improving flavour of fish oil for human consumption (Dinamarca and others 1990). Furthermore, it removes contaminants like chlorinated hydrocarbons, free fatty acids, oxidation products and cholesterol (Bimbo 1998). In this study, the distillation of the fish oil decreased the content of fatty acids (FFA) from 2.04% in the crude oil to 0.3%, and the AV decreased from 4.6 to 2.5.

Conjugated dienes and conjugated trienes

Absorbance around 232 nm is a measure of conjugated dienes (CD) which may result from decomposition of linoleic hydroperoxides (IUPAC 1987), an initial product of oxidation. This measurement has shown high correlation with other measurements of primary oxidation like peroxide value in olive oil triacylglycerols (Gómez-Alonso and others 2004), as well as with headspace oxygen in soybean oil (Chung and others 2004). Secondary products of autoxidation and, particularly ethylenic diketones, show an absorption band at approximately 268 nm together with conjugated trienes (CT) (IUPAC 1987). This measurement has shown good correlation with other measures of oxidation products as dimers and polymers of triacylglycerols from olive oil (Gómez-Alonso and others 2004). The CD values of mayonnaise in this study increased during the first week of storage from approximately 0.4 to 1.3-1.6 (Figure 1). Little difference was observed in CD-values between samples, although the control sample S (mayonnaise without fish oil) ended at a higher value of approximately 2, compared to 1.8 in the F sample, consisting of fish oil enriched mayonnaise without antioxidant addition ($P < 0.05$).

The CT values showed similar behaviour, with an incensement in the first week of storage, a lag phase between 1 and 3 weeks and a subsequent incensement (Figure 2). The values increased

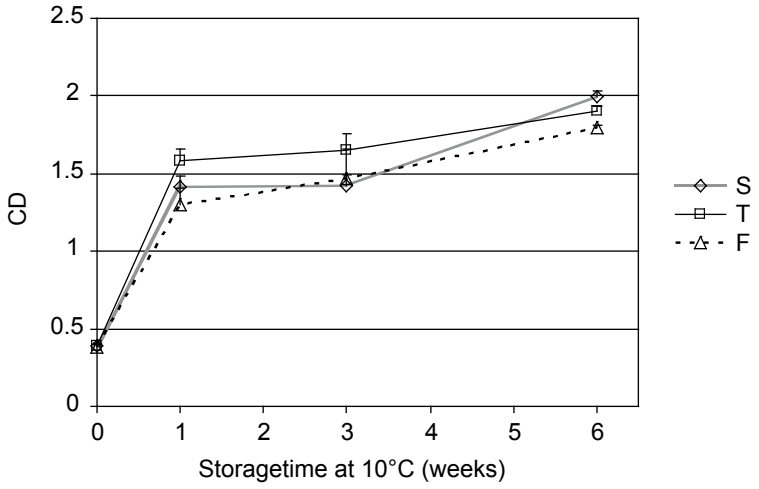


Figure 1. Changes in conjugated dienes (CD) during storage of mayonnaise (n = 2). S: mayonnaise with 100% soya oil, F: mayonnaise with soya and fish oil (80:20), T: same as F with tocopherol as antioxidant (200 mg/kg).

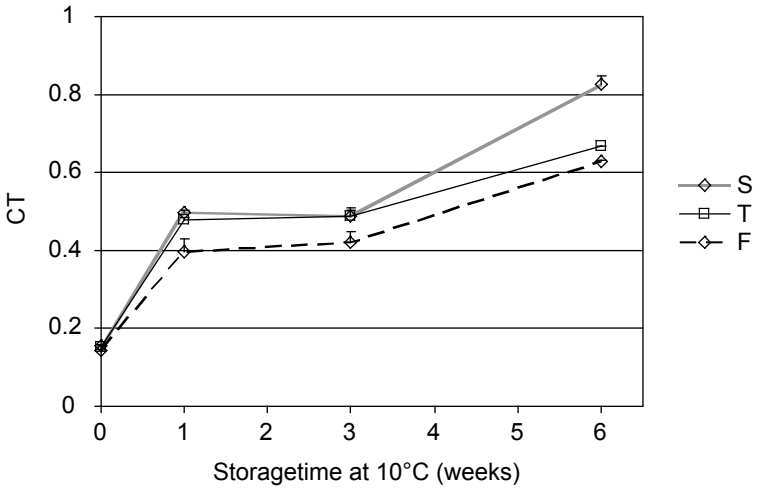


Figure 2. Changes in conjugated trienes (CT) during storage of mayonnaise (n = 2). For abbreviations, see Figure 1.

from approximately 0.15 to 0.6-0.8, and the control sample (S) had in fact higher CT values than the F sample after 1 and 6 weeks storage ($P < 0.05$). While the tocopherol addition (sample T) did not improve the stability of the fish oil mayonnaise as measured by CD or CT.

Sensory analyses

The "home made", egg-free mayonnaise prepared with diverse ingredients according to producer's recipe, contained 3.5% vinegar of 10% concentration, resulted in rather sour mayonnaise with pH of approximately 3.8 in all samples. The vinegar odour and flavour were also the most dominant sensory attributes for all samples, along with a thick and creamy texture (Table 2). The pH of emulsions has been found to play an important role for the stability of emulsions as the activity of proteins, such as whey proteins, has been found to be greatest at pH values below the p*I* of the proteins (Hu and others 2003). This effect has been attributed to the ability of the proteins to generate a positive electrical charge on the oil droplets, thereby repelling positively charged transition metal ions (McClements and Decker 2000). The same research team working with whey protein isolate at pH 3 found that it could be used effectively to protect polyunsaturated lipids from oxidation (Djordjevic and others 2004a, 2004b).

The colour of fish oil added mayonnaise (both F and T) was more yellow than soya oil mayonnaise (S) ($P < 0.05$). This colour difference between the two oils was obvious, soya oil being very pale yellow and the fish oil almost orange in colour, which resulted in a yellow mayonnaise in combination with soya oil, which alone produced almost white mayonnaise.

Odour attributes of mayonnaise revealed little differences between samples except for fish oil odour, where the S sample showed tendencies to be lower than the other samples, although only significantly lower than sample T after 6 weeks storage ($P < 0.05$). The S sample had values for fish oil odour ranging from 1 to 6, where as the F sample had values ranging from 11-22 and the T sample had values from 11 and reaching 27 at the end of the storage time (Table 2).

Table 2. Sensory scores (means, n = 2) for descriptive attributes of mayonnaise during storage on scale from 0-100 (not present to strong). Different superscripted letters indicate significant difference between samples within column (P < 0.05).

		Odour						Flavour						Texture			
Weeks	Sample*	vinegar	oily	musty	painty	fish oil	rancid	vinegar	oily	musty	painty	fish oil	rancid	thick	creamy	clammy	colour
0	S	60	9	4	8	1^{a}	1	55	17	10	9	0^{a}	4^{a}	64	66	19	13^{a}
	T	57	16	5	7	11	4	57	17	11	8	27^{b}	8	57	57	18	56^{b}
	F	57	16	5	7	11	4	57	17	11	8	27^{b}	8	57	57	18	56^{b}
1	S	57	20	2	4	4^{ab}	0	60	29	2	5	4^{a}	0	66	68	19	12^{a}
	T	56	18	2	10	15	3	57	21	6	11	30^{b}	6	64	58	20	59^{b}
	F	52	17	3	12	22^{bc}	3	51	22	6	12	31^{bc}	7	60	59	19	64^{b}
3	S	61	26	3	8	6^{ab}	2	58	30	9	13	9^{ac}	2	65	63	22	12^{a}
	T	57	18	10	13	13	5	60	23	9	19	28^{b}	11	67	66	28	53^{b}
	F	59	21	4	9	10	2	58	22	6	6	28^{b}	3	53	57	22	61^{b}
6	S	53	22	4	9	2^{a}	1	56	28	9	16	2^{a}	5	63	66	25	9^{a}
	T	55	16	6	11	27^{c}	8	58	22	12	13	33^{b}	16^{b}	65	65	24	57^{b}
	F	58	20	6	13	14	2	56	22	11	17	30^{bc}	6	56	58	23	54^{b}

*S: mayonnaise with soya oil, F: mayonnaise with soya and fish oil (80:20), T: same as F with tocopherol as antioxidant (200 mg/kg).

The score for fish oil flavour was higher for both F and T samples than the S sample throughout the storage time ($P < 0.05$). The fish oil flavour did however not increase in these samples during the storage, ranging from 0 to 9 in the S sample, but around 30 for the F and T samples throughout the storage time (Figure 3).

There was a more rancid tendency during storage of the T sample than in S sample ($P = 0.07$), ending in rancidity score of 16 for T but 5 for the S sample (Figure 4). The F sample was surprisingly more in range with the S sample, with rancidity scores from 3 to 8.

Comparable observations have been reported from studies with conventional egg yolk mayonnaise, where mayonnaise containing 16% fish oil did not oxidise faster than mayonnaise without fish oil, judged from chemical parameters such as peroxide value and anisidine values (Jacobsen and others 1999). However, unpleasant off-odours and off-flavours developed much faster in the fish oil enriched mayonnaise. The authors proposed that the fishy off-flavour compounds, in fish oil enriched mayonnaise might be caused by volatile compounds in trace amounts, with low sensory threshold, present in the water phase of the mayonnaise. That might explain why the oxidation was not higher in fish oil enriched mayonnaise, because the measurements were done on the lipid fraction and not in the water phase. Furthermore, the

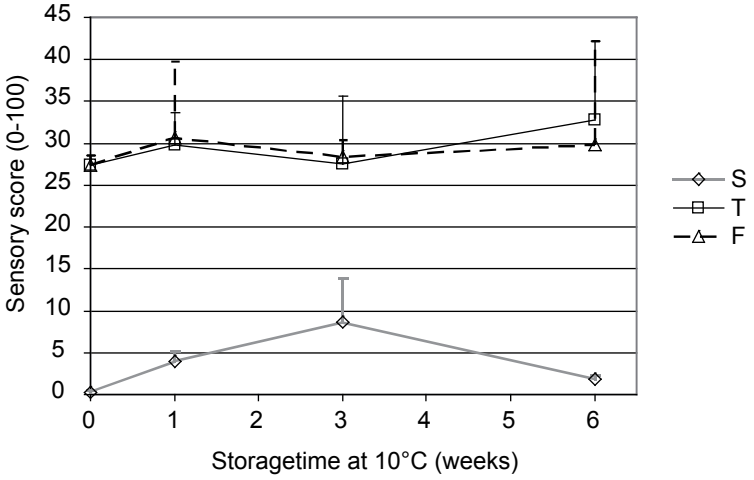


Figure 3. Evaluation of fish oil flavour by sensory panel (n = 2). For abbreviations, see Figure 1.

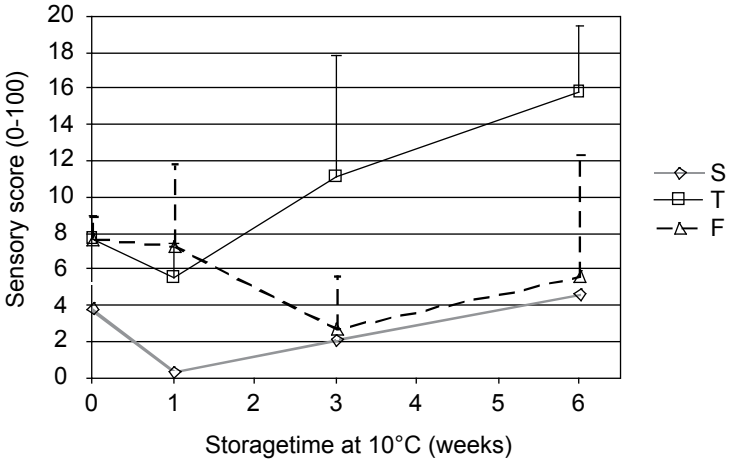


Figure 4. Evaluation of rancid flavour by sensory panel (n = 2). For abbreviations, see Figure 1.

measurement on volatile compounds may not be as sensitive to small amounts of off-flavour compounds as the sensory panel.

The tocopherol concentration chosen in this study (200 mg/kg of mixed tocopherols) was the same as conventional addition to cod liver oil, in the fish oil industry. The tocopherol addition did not improve the stability or the sensory quality of the mayonnaise in this study, but showed tendencies to induce rancidity during storage according to sensory evaluation. These results were in fair agreement with the work of Jacobsen and others (2000) who found no difference in the development of volatile off-flavours as evaluated by GC-MS, but the peroxide values were slightly increased in tocopherol (200 mg/kg) added mayonnaise. The sensory perception of the

fish oil enriched mayonnaise was on the other hand not affected by the tocopherol addition in their study. Likewise, tocopherol did not appear to be an efficient antioxidant in another study with both water-dispersible tocopherols and oil-soluble tocopherols in 20-280 mg/kg concentrations added to 16% fish oil enriched mayonnaise (Jacobsen and others 2001). In a study with fish oil enriched milk emulsion, addition of a tocopherol mixture did not increase the stability of the milk emulsion, while rapeseed oil containing natural tocopherols in combination with fish oil, inhibited oxidation (Let and others 2005). Research with microencapsulated fish oil, produced in emulsions with coating materials and microencapsulated by spray drying, showed that samples containing DL-α-tocopherol (1000 mg/kg) were more stable than unprotected samples (Jónsdóttir and others 2005). These results indicate that addition of tocopherols to fish oil emulsions is a delicate balance between antioxidative and pro-oxidative factors, and the content of endogenous tocopherols in the lipids needs to be considered.

Comparison of testing methods

Stability test of mayonnaise samples at 60 °C in Oxipres revealed very little difference between samples, as the pressure drop occurred at almost the same time in all samples (results not shown).

The results from the chemical measurements (CD and CT), as well as the Oxipres stability test, indicated that there was little difference in the oxidative stability of the control soya oil mayonnaise and the fish oil enriched mayonnaise. In fact, the results give the impression that the fish oil enriched mayonnaise was slightly more stable during storage. The sensory analysis gave on the other hand clear message; the enrichment of fish oil into mayonnaise could be detected by fish oil flavour (and odour) with sensory scores around 4 for the control mayonnaise and 30 (on a scale from 0–100) for fish oil enriched mayonnaise. Rancid flavour was more pronounced already in the freshly made fish oil enriched mayonnaise, which indicates that it might have been confused with the fish oil flavour, which remained essentially stable throughout the storage experiment. The capelin oil used in this study was only refined by molecular distillation in lab-scale in order to use as few intervening processing steps as possible in order to maintain endogenous antioxidants and minimise oxidation. Because processes like bleaching and deodorisation cause major loss of retinols, tocopherols and other constituents with antioxidant activity as well as health beneficial effects (Scott and Latshaw 1991; Dunford 2001). As a result, the fish oil flavour might have been more pronounced than by conventional fish oil production involving alkali refinement, bleaching and deodorisation. More optimal conditions regarding refinement of the capelin oil, concentration of the capelin oil as well as incorporation of the mayonnaise into some tasteful seafood, are only to mention few of the factors that could be investigated in order to verify if capelin oil could be generously applied for human consumption.

Conclusions

The focus in this preliminary study was to investigate the use of capelin oil in mayonnaise, with emphasis on sensory acceptance. The lab-scale refinement of capelin oil and production of mayonnaise replacing 20% of soya oil with capelin oil resulted in a mayonnaise with a significant fish oil flavour. Lipid oxidation of the mayonnaise was not increased by fish oil addition although sensory scores for rancidity were higher for the fish oil enriched mayonnaise that contained tocopherol as antioxidant. Tocopherol was therefore ineffective as antioxidant in the fish oil enriched mayonnaise using 200 mg/kg of mixed tocopherols, but tended to increase

oxidative degradation in this study. Optimisation of processing parameters by studies on the effect of raw material qualities and refinement techniques are necessary in order to conclude if capelin oil can be used in functional foods with adequate sensory acceptance. Potential possibilities are, however, in sight for capelin oil, as this study indicated that acceptable stability regarding lipid oxidation could be obtained.

Acknowledgements

We gratefully thank the AVS fund for financial support, Guðjón Rúnarsson at Kjarnavörur Ltd for advice and for providing the soya oil, Þórhallur Jónasson at SVN Ltd for his cooperation and for providing the fish oil, Danisco A/S for providing the Grindsted stabiliser system and Jón Ögmundsson and Eiríkur Kristinsson at Lýsi Ltd for their collaboration. Special thanks to the sensory panellists of IFL for their generous contribution.

References

AOAC. 1990. Official methods of analysis, 15th ed. Arlington, Virginia: Association of Official Analytical Chemists.

AOCS. 1998. Official methods and recommended practices of the American Oil Chemists Society. 5th ed. Champaign, Illinois: American Oil Chemists´ Society.

Bimbo, AP. 1998. Guidelines for characterizing food-grade fish oil. Inform 9(5):473-483.

Bragadóttir M, Pálmadóttir H, Kristbergsson K. 2002. Seasonal changes in chemical composition and quality parameters of capelin (*Mallotus villosus*). J Aquatic Food Prod Technol 11(3/4):87-103.

Bragadóttir M, Þórisson S, Hjaltason B. 1992. Þránun Lýsis. Rannsóknastofnun fiskiðnaðarins. Rit Rf 32:1-35.

Chung HJ, Colakoglu AS, Min DB. 2004. Relationships among headspace oxygen, peroxide value, and conjugated diene content of soybean oil. J Food Sci 69(2):FTC83-FTC88.

Coupland JN, McClements DJ. 1996 Lipid oxidation in food emulsions. Trends Food Sci Technol 7:83-91.

Dinamarca E, Garrido F, Valenzuela A. 1990. Simple high vacuum distillation equipment for deodorizing fish oil for human consumption. Lipids 25(3):170-171.

Djordjevic D, Kim HJ, McClements DJ, Decker EA. 2004a. Physical stability of whey protein-stabilized oil-in-water emulsions at pH 3: Potential ω-3 fatty acid delivery systems (Part A). J Food Sci 69(5):C351-C355.

Djordjevic D, McClements DJ, Decker EA. 2004b. Physical stability of whey protein-stabilized oil-in-water emulsions at pH 3: Potential ω-3 fatty acid delivery systems (Part B). J Food Sci 69(5):C356-C362.

Donnelly JL, Decker EA, McClements DJ. 1998. Iron-catalyzed oxidation of menhaden oil as affected by emulsifiers. J Food Sci 63(6):997-1000.

Dunford NT. 2001 Health benefits and processing of lipid-based nutritionals. Food-Technol 55(11):38, 40-44.

Gómez-Alonso S, Salvador MD, Fegapane G. 2004. Evolution of the oxidation process in olive oil triacylglycerol under accelerated storage conditions (40-60°C). J Am Oil Chem Soc 81(2):177-184.

Hu M, McClements DJ, Decker EA. 2003. Impact of whey protein emulsifiers on the oxidative stability of salmon oil-in-water emulsions. J Agric Food Chem 51:1435–1439.

ISO. 1993. Sensory analysis - general guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors, 8586-1. Genf, Switzerland: The International Organization for Standardization.

IUPAC. 1987. Standard Methods for the Analysis of Oils, Fats and Derivatives. 7th ed. Oxford, UK: Blackwell Scientific Publications.

Jacobsen C, Hartvigsen K, Lund P, Meyer AS, Adler-Nissen J, Holstborg J, Hølmer G. 1999. Oxidation in fish-oil-enriched mayonnaise. 1. Assessment of propyl gallate as an antioxidant by discriminant partial least squares regression analysis. Eur Food Res Technol 210:13-30.

Jacobsen C, Hartvigsen K, Lund P, Meyer AS, Adler-Nissen J, Holstborg J, Hølmer G. 2000. Oxidation in fish-oil-enriched mayonnaise. 2. Assessment of the efficacy of different tocopherol antioxidant systems by discriminal partial least squares regression analysis. Eur Food Res Technol 210:242-257.

Jacobsen C, Hartvigsen K, Lund P, Thomsen MK, Skibsted LH, Hølmer G, Adler-Nissen J, Meyer AS. 2001. Oxidation in fish oil-enriched mayonnaise: 4. Effect of tocopherol concentration on oxidative deterioration. Eur Food Res Technol 212:308-318.

Jónsdóttir R, Bragadóttir M, Arnarson GÖ. 2005. Oxidatively derived volatile compounds in microencapsulated fish oil monitored by solid-phase microextraction (SPME). J Food Sci 70(7):C433-C440.

Kolanowski W, Swiderski F, Berger S. 1999. Possibilities of fish oil application for food products enrichment with omega-3 PUFA. Int J Food Sci Nutr 50:39-49.

Let MB, Jacobsen C, Pham KA, Meyer AS. 2005. Protection against oxidation of fish-oil-enriched milk emulsions through addition of rapeseed oil or antioxidants. J Agric Food Chem 53:5429-5437

McClements DJ, Decker EA. 2000. Lipid oxidation in oil-in water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J Food Sci 65:1270-1282.

Medina I, González MJ, Pazos M, Medaglia DD. 2003. Activity of plant extracts for preserving functional food containing n-3-PUFA. Eur Food Res Technol 217(4):301-307.

Schmidt EB, Arnesen H, De Caterina R, Rasmussen LH, Kristensen SD. 2004. Marine n-3 polyunsaturated fatty acids and coronary heart disease: Part 1. Background, epidemiology, animal data, effects on risk factors and safety. Thromb Res 115(3):163-170.

Scott KC, Latshaw JD. 1991 Effects of commercial processing on the fat-soluble vitamin content of menhaden fish oil. J Am Oil Chem Soc 68(4):234-236.

Simopoulos AP, Cleland LG. 2003 Omega-6/omega-3 essential fatty acid ratio: The scientific evidence. Basel, Switzerland: Karger AG. 174 p.

Stone H, Sidel JL. 1985. Sensory evaluation practices. Orlando, Florida: Academic Press. 311 p.

Uauy R, Valenzuela A. 2000. Marine oils: The health benefits of n-3 fatty acids. Nutrition 16:680-684.

Young V. 1990. The usage of fish oils in food. Lipid Technol 2(1):7-10.

Oxidative stability of fish oil enriched yoghurts

Charlotte Jacobsen[1], Mette B. Let[1], Gitte Andersen[1] and Anne S. Meyer[2]
[1]Department of Seafood Research, Danish Institute for Fisheries Research, B.221, DTU, DK-2800 Kgs. Lyngby, Denmark
[2]BioCentrum-DTU, B.221, DTU, DK-2800 Kgs. Lyngby, Denmark

Abstract

In this study the oxidative deterioration of fish oil enriched yoghurt with or without strawberry jam was investigated. Two different emulsifiers (citric acid ester or milk protein) were evaluated. Moreover, the oxidative stability of yoghurts prepared with pure fish oil or a mixture of fish oil and rapeseed oil was compared. The sensory off-flavour, concentration of lipid hydroperoxides and volatile secondary oxidation products, determined by dynamic headspace GC/MS, were monitored during cold storage. Fish oil enriched yoghurt only oxidised slightly during storage. Yoghurts prepared with a mixture of fish oil and rapeseed oil were more stable than yoghurts prepared with pure fish oil. The choice of emulsifier did not seem to affect oxidative stability.

Keywords: omega-3 fatty acids, rapeseed oil, yoghurt, volatile oxidation products, sensory analysis

Introduction

A still increasing amount of evidence supports the proposed beneficial health effects of the marine n-3 long chain polyunsaturated fatty acids (PUFA) (Kris-Etherton and others 2002; Hardman 2002; Trebble and others 2003). The two most potent n-3 PUFA seem to be C20:5 n-3 (EPA) and C22:6 n-3 (DHA). The most well documented effect is the apparent ability of these n-3 PUFA to protect against cardiovascular death (Kris-Etherton and others 2002). Recently, a high intake of n-3 PUFA has also been associated with lower risk of developing Alzheimer and depressions (Mischoulon and Fava 2000). Marine oils are rich sources of EPA and DHA. Therefore, many efforts have been made to incorporate marine oils into various food products. Several functional food products based on dairy ingredients such as probiotic yoghurts are already on the market and these products are perceived as being healthy by the consumers. Therefore, yoghurts and other dairy products may be good vehicles for incorporation of n-3 PUFA into food products. However, due to their highly polyunsaturated nature marine oils are very susceptible to lipid oxidation, which will lead to the formation of unhealthy compounds and undesirable fishy and rancid flavours.

Previous studies have dealt with the oxidative stability of fish oil enriched milk and different strategies to reduce lipid oxidation were investigated (Let and others 2003, 2004, 2005a and b). These studies showed that the otherwise significant lipid oxidation in fish oil enriched milk could be prevented by using a mixture of fish oil and rapeseed oil instead of using pure fish oil (Let and others 2004, 2005b).

The rate of lipid oxidation in complex food emulsions depends on numerous factors (Frankel 2005; Jacobsen 2004). Several studies in simple oil-in-water emulsions have suggested that

the choice of emulsifier used to prepare the emulsion can significantly affect lipid oxidation (Mei and others 1999; Hu and others 2003). Thus, Mei and others (1999) suggested that positively charged emulsifiers/surfactants could reduce oxidation, because positively charged metal ions would be repelled from the oil-water interface where they would otherwise catalyze oxidation. In contrast, negatively charged surfactants were suggested to increase oxidation. Recently, Djordjevic and others (2004) suggested that fish oil-in-water emulsions prepared with whey proteins as emulsifiers could be used as n-3 PUFA carriers to be incorporated into food products.

Lipid oxidation of n-3 PUFA rich products give rise to the formation of highly undesirable fishy and rancid flavours (Jacobsen 1999; Let and others 2003). This flavour is extremely penetrating in fish oil enriched milk, because milk in it-self has a mild flavour that cannot mask the fishy flavour. The volatiles formed in fish oil enriched milk during oxidation has previously been characterised (Venkateshwarlu and others 2004a). Later, four compounds, namely 1-penten-3-one, 2,4-t,t-heptadienal, c-4-heptenal and 2,6-t,c-nonadienal, were suggested to play a significant role for the intensity of fishy and metallic flavours in fish oil enriched milk (Venkateshwarlu and others 2004b). Yoghurt products are often flavoured with fruits and it is likely that the fruity taste could mask fishy flavours. Moreover, berries used in fruit jams (*e.g.* strawberry, blueberry) have been shown to have antioxidative properties (Klopotek and others 2005). The effect of antioxidants in lipid systems has been suggested to depend on their polarity. According to the so-called "polar paradox" polar antioxidants such as trolox will be more effective in non-polar systems like bulk oils while less polar antioxidants such as tocopherol will be more effective in oil-in-water emulsions (Huang and others 1996).

The objective of this study was therefore to determine the oxidative stability of strawberry flavoured yoghurt enriched with fish oil. A second objective was to investigate if yoghurt based on a mixture of rapeseed oil and fish oil would be less susceptible to oxidation than yoghurt based on pure fish oil. Oils were added to the yoghurt in the form of an oil-in-water emulsion. The effect of using two structurally very different emulsifiers to prepare this oil-in-water emulsion was also investigated. A final objective was to investigate whether the addition of strawberry jam to fish oil enriched yoghurt affected its oxidative stability compared with yoghurt without strawberry jam.

Materials and methods

Fresh non-flavoured yoghurt with a fat content of 1.5 wt % was purchased locally. Refined cod liver oil as well as refined rapeseed oil without added antioxidants were provided by Maritex A/S, Århus, Denmark. A mixture of cod liver oil and rapeseed oil (1:1) was deodorised at Biocentrum-DTU, Technical University of Denmark, Lyngby, DK, as previously described (Let and others 2004). Data on oils are shown in Table 1. The fatty acid composition was determined by preparation of methyl esters that were in turn analyzed by gas chromatography (AOCS Official method Ce 2-66) The levels of tocopherols were determined by HPLC (AOCS Official method Ce 8-89). Tocopherol standards were purchased from Calbiochem, San Diego, CA. Chemicals and external standards for identification of volatile oxidation products were all purchased from Sigma Aldrich, Steinheim, Germany. All solvents were of HPLC grade from Lab Scan, Dublin, Ireland. Citric acid ester, Citrem LR10 extra (based on glycerolmonooleate) was kindly donated from Danisco (Brabrand, Denmark) from and milk protein Nutrilac D8080 was from Arla Foods Ingredients (Aarhus, Denmark).

Table 1. Data on oils used for experiment 1 and 2.

	Fish oil 1[a]	Rapeseed/fish oil mixture	Rapeseed oil	Fish oil 2[a]
PV (meq/kg)	0.10	0.64	1.43	2.0
Free fatty acids (%)	0.05	0.01	0.02	0.01
Alpha-tocopherol (mg/kg)	312	244	159	330
Beta-tocopherol (mg/kg)	ND	33	71	ND
Gamma-tocopherol (mg/kg)	3.6	158	344	NA
Delta-tocopherol (mg/kg)	16.4	3.4	7	NA
14:0	3.7	1.9	0.0	3.5
16:0	9.8	6.9	4.2	10.3
18:0	2.0	1.8	1.7	2.3
Sum SFA	15.5	10.6	5.8	16.1
16:1 (n-7)	6.3	3.2	0.2	6.7
18:1 (n-9)	16.7	37.3	57.6	18.9
18:1 (n-7)	3.7	3.3	2.8	4.4
20:1 (n-9)	12.2	6.2	1.4	14.3
22:1 (n-11)	7.1	3.7	ND	8.7
Sum MUFA	46.0	53.6	62.0	53.0
18:2 (n-6)	1.7	10.2	18.6	1.9
18:3 (n-3)	0.9	4.6	8.3	1.1
18:4 (n-3)	2.7	1.3	ND	3.0
20:5 (n-3)	8.6	4.2	ND	9.7
22:5 (n-3)	1.0	0.5	ND	1.2
22:6 (n-3)	11.9	5.7	ND	13.4
Sum PUFA	26.8	26.5	27.0	30.3

[a] Fish oil 1 was used in experiment 1 and Fish oil 2 was used in experiment 2, ND = not detectable, NA = Not analysed.

Production of pre-emulsion for yoghurt

The fish oil was added to the yoghurt as a pre-emulsion. This oil-in-water emulsion (71% oil) was prepared in a Stephan Mixer (Hameln, Germany). Different procedures were used for the production of the emulsions based on citric acid and milk protein, respectively. In case of the emulsion with citric acid, 360 g oil was heated and stirred in a beaker and 4.051 g citric acid was slowly added. The heating was continued until a temperature of 65 °C was reached. 144 g distilled water was poured into a Stephan mixer pre-heated by hot water. Subsequently, the oil-emulsifier mixture was gradually (3 min.) added to the water while stirring at a speed of 1200 rpm. Then the stirring speed was increased to 1500 rpm and stirring was continued for another 2 ½ min. In the milk protein emulsion the amount of emulsifier (Nutrilac D-8080) was 5.401 g and the emulsifier was manually mixed with water in the Stephan mixer bowl. The emulsification of the water-emulsifier mixture with the oil was as described above for citric acid.

Production of yoghurt

Yoghurts were prepared both with and without strawberry jam in accordance with the experimental plan in Table 2a and b. In case of strawberry-flavoured yoghurt, 14.0 g emulsion was added to 136.0 g strawberry jam in a beaker and mixed by hand. The strawberry-oil emulsions was subsequently mixed into 850.0 g yoghurt by hand. This yoghurt contained 2.27% fat of which 0.99% was from the oil added and 1.28% was from milk fat. In case of the non-flavoured yoghurt, 14.0 g emulsion was added directly to 986.0 g yoghurt and mixed as previously described. This yoghurt contained 2.47% fat of which 0.99% was from the oil added and 1.48% was from milk fat.

Storage experiment and preparing samples for analysis

Yoghurts from experiment 1 were stored in 1 L blue cap bottles while yoghurts from experiment 2 were stored in 250 mL blue cap bottles. In both experiments yoghurts were stored at 5 °C in the dark for up to four weeks. Yoghurts were subjected to sensory evaluation (only experiment 1), peroxide value (PV) determination, and dynamic headspace GC-MS analyses. As for the yoghurts from experiment 1, samples for chemical analysis were transferred to separate 250 mL blue cap bottles, flushed with nitrogen, and stored at –80 °C until analyses, while samples for sensory analyses were evaluated directly at sampling. Bottles from experiment 2 were transferred directly to the –80 °C freezer after sampling and kept at this temperature until chemical analyses were performed.

Analyses of primary oxidation products

Yoghurts were thawed and 15 g sample was taken for lipid extraction by chloroform:methanol (1:1 w/w) using a reduced amount of solvent (Iverson and others 2001; Bligh and Dyer 1959) PV was measured directly on the oils or on the fat extract from the yoghurt by colorimetric determination of iron-thiocyanate (International IDF Standard 103A:1986).

Dynamic headspace analysis of volatile secondary oxidation products

Volatile secondary oxidation products from 10.0 g of yoghurt were purged and trapped on Tenax GR® tubes with nitrogen (150 mL/min) for 30 min at 50 °C using 4-methyl-1-pentanol as internal standard. The volatiles were desorbed (200 °C) from the trap in an automatic thermal desorber (ATD-400, Perkin Elmer, Norwalk, CN) and cryofocused on a Tenax GR cold

Table 2a. Design of experiment 1.

	Pure rapeseed oil	Mixture of fish oil and rapeseed oil (1:1)	Pure fish oil
Milk protein	ROMP	RFMP	FOMP
Citric acid ester	ROCE	RFCE	FOCE

Table 2b. Design of experiment 2.

	With strawberry jam	Without strawberry jam
Milk protein	StrawMP	MP
Citric acid ester	StrawCE	CE

trap. Volatiles were separated by gas chromatography (HP 5890 IIA, Hewlett Packard, Palo Alto, CA) and analyzed by mass spectrometry (HP 5972 mass-selective detector). Oven temperature programme: 45 °C held for 5 min, 1.5 °C/min to 55 °C, 2.5 °C/min to 90 °C, 12 °C/min to 220 °C and finally held at 220 °C for 4 min. The individual compounds were identified by both MS-library searches (Wiley138K, John Wiley and Sons, Hewlett Packard, US) and by authentic external standards. The individual compounds were quantified through calibration curves.

Sensory evaluation

Yoghurts were evaluated by descriptive analysis by 9-13 panelists trained in descriptive analysis of yoghurt samples with fishy off-flavours. ISO standards 6658, 8586, and 6564 were generally followed for training and sensory analysis methods, respectively. The descriptors used for odour and flavour assessment were fishy, rancid, astringent/yoghurt, strawberry/sweet and others, and these were evaluated on a continuous intensity scale ranging from zero intensity to a maximum intensity of 9. Samples (25 mL) were served randomized at 5 °C with crisp bread and cold water in blind trials after 1, 8, 12 and 22 days of storage. Data were collected on PSION mini computers (PSION, London, UK).

Statistical analysis

Experiment 1

Sensory data were not normaly distributed and could therefore not be analysed by a two-ways ANOVA analysis. Instead, Kruskal Wallis test was used. In addition, all data were analysed by ANOVA Partial Least Squares Regression (APLSR) analysis using the software programme Unsrambler version 7.6 (Oslo, Norway). Design variables (Rapeseed, Rapeseed and fish, Fish oil for oil types and Emulsifier type) were used as X-data and the measured variables (sensory data, PV and volatiles) were used as Y-data. Only volatiles that seemed to change during storage were included in the model (nonanal, pentanal, hexanal, t-2-hexenal, t,t-2,4-heptadienal, penten-3-one, penten-3-ol). Likewise, only those sensory variables, which were significantly different among the 6 yoghurts as evaluated by a Kruskal-Wallis test ($p < 0.05$) were included in the APLSR model (fishy odour and flavour, rancid odour and flavour, sweet odour and flavour). A so-called long thin data matrix was used for the model, where the yoghurts at each sampling point was used as subjects and only one variable for each type of sensory descriptor/PV/volatile compound was used. All data were weighted by 1/standard deviation and full cross validation was used to validate the model. By using the jack knifing facility in Unscrambler it was possible to calculate the regression coefficients for the variables.

Experiment 2

PV and volatiles data were analysed by APLSR analysis as described above. Only volatiles that developed during storage and that seemed to differ between the different samples were included in the model (2-ethylfuran, 2-pentanone, 2-t-hexenal, 1-hexanol, 2-heptanol, hexanal, 2-heptanone, hexanoic acid, 2-nonanone, octanoic acid). Design variables used in this experiment were yoghurt type and emulsifier type.

Results and discussion

Experiment 1

In experiment 1, the oxidative stability of strawberry flavoured yoghurts produced from different oil types and two different emulsifiers was assessed by sensory evaluation and analysis of peroxide values (PV) and volatile secondary oxidation products.