Chapter 1

GENERAL INTRODUCTION

The difference between fish oil and other oils is mainly due to the unique variety of fatty acid it contains. Thus, marine fish oils are excellent sources of polyunsaturated fatty acids (PUFAs), mainly long-chain omega 3 fatty acids, also sometimes referred to as n-3 fatty acids. The amount and variety of the fatty acids in fish oil varies from one fish species to another, and also with the biological stage, food availability, fishing location, ocean temperature, nutritional and spawning state, etc. The major omega 3 fatty acids present are the eicosa pentaenoic acid (C20:5 omega-3, commonly called EPA) and the docosa hexaenoic acid (C22:6 omega-3, commonly entitled DHA). These Long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs), have long been proposed to bestow health benefits by improving blood pressure control, alleviating symptoms of rheumatoid arthritis and depression, as well as attenuating the progression of Alzheimer’s disease. Marine algae and its feeder fishes are the richest source of omega 3 fatty acids, which are essential to human metabolism. Poly unsaturated fatty acids (PUFAs) cannot be synthesized in our body, but it can be synthesized by elongase and desaturase pathway from the precursor fatty acids. This particular pathway is observed in marine planktons.

Health benefits of PUFA attribute to prevention of cardio vascular disease, reduction of the clinical onset of cancer cells, rejuvenation of the brain cells and retinal cells in infants, prevention of rheumatoid arthritis etc. The USFDA (United States Food and Drug Administration) recommends daily
requirement of PUFA as 5.0 mg/day, which is genuinely supplied from the marine sources. Hence most of the nutraceutical companies exploit fishery resources in making fish oil capsules. The best strategy to increase the intake of long chain \(n\)-3 PUFAs and to increase the content of long chain \(n\)-3 PUFAs in blood, cells and tissues is to eat oily fish regularly. An alternative is to consume fish oil capsules, which is a highly effective way of increasing long chain \(n\)-3 PUFA status in human blood and cells. It is apparent that foods either naturally enriched or fortified with long chain \(n\)-3 PUFAs will become increasingly available over the next 5 years and this will be a very good strategy to increase intake of these fatty acids, and will provide greater choice for those consumers who do not eat fish and who do not wish to take capsules.

The reliability and the stability of these PUFA enhancers is a matter of concern, since the deteriorated fish oil supplements adversely affect health. Hence studies are required emphasizing the need to exploit alternate sources of PUFA also other than fish oil. It can be a primary producer in ocean’s comprising chiefly algae. Since the sustainability of fish resource is a matter of concern and based on fishery resource management data, we cannot rely fully on fish as a sole source of PUFA. There by, the utilization of fish in energy rich supply of omega 3 fatty acid need to be reduced and instead, the exploitation of marine algae need to be stabilized. Enrichment of PUFA by bio processing such as urea crystallization, super critical fluid extraction methods, lipase catalyzed reaction method, molecular distillation etc. are employed widely to meet this requirement.

There is a wide range of species used in the production of fish oils. However, the biggest part derives mostly from the fatty fishes and the liver of lean fishes. The commonly used raw materials are menhaden, sardines, anchovies, herring, capelin, mackerel, salmon, tunas, cod liver, etc. Besides
from bony fishes, fish oil and fish liver oil can be derived from cartilaginous fishes like sharks. Shark and cod are the two main fish whose liver are extracted for oil. While sharks are specifically targeted for their livers, cod livers are taken as a by-product along with the roe. Tropical marine fatty fishes which include mackerel, tuna and sardines contain high level of poly unsaturated fatty acids. Among these, considering the availability and economic cheapness, sardines could be selected as the best source of fish oil supplements for production of omega 3 fatty acids. It contributes to more than 40% of the available total lipid content from marine fishes. Seasons and ocean currents affect the life cycle pattern of all species surviving in the marine ecosystem. These overall changes affect the individual inhabitants which also influences the lipid content.

A thorough knowledge about the variation in lipid in sardines is much needed for understanding the availability of PUFA in various seasons. Many of the experts in the fishery field have determined the proximate composition as well as seasonal variation in lipid content of Sardines earlier (Gopakumar and Rajendranathan, 1975). Climate change is also likely to play an important role in the future of fishing (Lam et al., 2012), while ocean warming may increase productivity for some stocks, ocean acidification and warming (Voss et al., 2015) generally decrease the productivity of stocks.

Most of the exporting companies depend on the freezing method of storage of sardines (Sardinella longiceps). Even though, this method of fish preservation is effective, there can be degradation occurring, thus affecting the quality of fish on prolonged storage. The indices for deterioration show that lipid oxidation steadily increases with the storage days even in frozen fishes.
Along with the quality of fatty fishes exported, the health effect of consumers is also a serious matter of concern. Lipid oxidation produces free radicals such as super oxide hydroxyls which can be even more dangerous causing human cancers. Research data in this regard could be useful for formulating the storage period of fatty acids in industries which uses these fatty acids for manufacture of PUFA. The onset of primary oxidation products may not be ideal neither for exporting nor for human consumption as it can alter adversely the metabolism in the consumers.

The highly perishable nature of the fatty fishes leads to search for an alternative source for PUFA. The suggested source is its primary producers in ocean ecosystem ie; marine algae. Countries like Japan, Taiwan, Indonesia etc. culture, harvest, utilize and incorporate marine algae in their table served foods (Molina-Grima et al., 2015). These are rich in PUFA and the synthesis of fatty acids could be dependent on culture conditions such as luminance, oxygen availability, and nutrient supply. As the culturing days progress, the bio mass increases and the utilization of nutrients increases. This will be propagated with variations in lipid content. Constant decline implies the death phase of the algae that differ widely among different species of algal culture. The growth curve of many of the algae had been determined, but lack of correlation with the lipid content made it difficult to predict the PUFA contents.

It is important to stress that there are numerous alternative methods for the concentration of omega-3 fatty acids, but only a few are suitable for large-scale production, the most widespread process being the concentration of omega-3 fatty acids by molecular distillation. Other available methods less commonly used include adsorption chromatography, enzymatic splitting, low-temperature crystallization, supercritical fluid extraction, and urea complexation. Each methodology has its own advantages and drawbacks.
Concerning enzymatic techniques, lipases are utilized. These enzymes can catalyze esterification, hydrolysis or exchange of fatty acids in esters (Marangoni and Rousseau, 1998). Accordingly, this technology may enable the performance of the trans esterification and concentration phases in a single step. The direction and efficiency of the reaction can be influenced by the choice of experimental conditions (Yadwad et al., 1991). PUFA Bio-enrichment by lipase enzyme is found to be eco-friendly as well as consumer friendly. The region-specificity of this enzyme will act on saturated bonds and will get cleaved, while the unsaturated double bond containing PUFA will not be cleaved due to hindrance effect. This result in the enrichment of PUFA and many studies had been carried out in fish oil samples. This information could provide nutraceutical companies in providing vegetative as well as non-vegetative PUFA capsules as enriched products replacing ordinary fish oils.

Value added food products can also be prepared using enriched PUFA. There are reports in which marine algae in powdered form are applied in biscuits, bread etc. and these can be replaced by enriched PUFA (Gouveia et al., 2008). Thus more specifically, health beneficial, nutritional supplements can be introduced directly into the food products.

Storage and packaging of refined fish oils are essential to preserve omega-3 PUFA from oxidation. Several studies have been published in recent years that have assessed the oxidative quality and fatty acid content of encapsulated and liquid formulations of EPA/DHA. When some studies concluded that most tested products were defective (Lee et al., 2016, Nichols et al., 2016) other studies have reported a significant proportion of tested products failing one or more quality parameters (Kleiner et al., 2015, Ritter et al., 2013).
In addition, other strategies to improve fish oil stability and extend its shelf-life are necessary (Kamal-Eldin and Yanishlieva, 2002). For that reason, the use of antioxidants is a common method to preserve refined fish oil, preventing its oxidation. A great number of antioxidants, such as butylated hydroxyanisole (BHA), butylatedhydroxytoluene (BHT), ethyldenediaminetetraacetic acid (EDTA), tocopherols, ascorbic acid, ascorbylpalmitate, propylgallate, gallicacid, lactoferrines, and others, have been tested to prevent lipid oxidation both in bulk oil (Kamal-Eldin and Yanishlieva, 2002) and in fish oil-in-water emulsions (Jacobsen et al., 2008). In the last decade, the use of natural antioxidants instead of synthetic compounds has received increasing attention and several studies regarding the efficiency of plant extracts, as oregano, parsley or rosemary, on the stabilization of bulk fish oil and fish oil-in-water emulsions have been carried out (Frankel et al., 1996; Bhale et al., 2007; Jiménez-Álvarez et al., 2008).

The adequate concentration of antioxidant depends on the chosen substance, the storage conditions and specific applications for the omega-3 PUFA concentrates. Antioxidants are substances which are effective in preventing propagation of oxidation by terminating the progress by capturing free radicals to produce a stable product. Food and Drug Administration (FDA) has stipulated the use of synthetic antioxidants and many experts rely on natural antioxidants. There are many natural antioxidants present in plant habitat which has been used widely till now. Vitamin E is a natural antioxidant present in fruits and green leaves. The phenolic components present in various plant species are identified and applied as natural antioxidants. Herbs and spices contain rich source of antioxidants which are primarily used as an effective preservative. Moreover the medicinal value of these plants is well known and is written in ancient Ayurveda scriptures. Preservation using natural antioxidants...
from the selected herbs is highlighted as an objective which makes the study very relevant for preserving products enriched with PUFA.

**Objectives of the Study**

The main objectives of the thesis are as follows:

- To analyze the seasonal variation of PUFA of fish oil extracted from *Sardinella longiceps*
- To identify and analyze PUFA in the marine algae such as *Isochrysis galbana, Chaetocerous calcitrans, Tetraselmis gracillus* and *Chlorella marina* as primary sources.
- To concentrate PUFA in fish oil and marine algae using microbial lipase from *Candida cylindracea*.
- To enhance the oxidative stability of PUFA in fish oil by incorporating Indian Medicinal Plant extracts of *Aloe barbadensis, Boherravia diffusa* and *Osimum sanctum*.