The issues that plague world health have swerved with the times. New pharmaceutical drugs, research into the lifecycle of pathogens, disease prevention and vaccination have helped eradicate major health risks posed by infections, and many diseases are believed to be eradicated. The report of World Heath Organisation (WHO) on global patterns of health risks renders plenty of evidence that the major health risks we face today do not lie with any particular infection but are the consequences of an unhealthy lifestyle and perverted eating habits.
As stated in the report of WHO, the top five contributors to world’s health problems are high blood pressure, tobacco use, high blood glucose, physical inactivity, overweight and obesity. Next up on the list is high cholesterol. These factors are responsible for raising the risk of chronic diseases, such as heart disease and cancers. They affect countries across all income groups: high, middle and low. Internationally, health experts are now asserting the importance of a healthy lifestyle that involves a conscientious diet and meticulous exercise regimen. ‘Prevention is better than cure’ as the saying goes.

The food that traditionally forms the staple of the local diet goes far in ensuring the health of a population - preventing the onslaught of the said maladies, and exerting a positive influence where they have made their ominous presence already felt. It is no secret that the longevity and wellness enjoyed by the Japanese, particularly Okinawans, are a result of their centuries-old dietary habits which include plenty of seaweed and fish. On an average, a Japan national consumes half a pound of fish each day. Japanese women also command the lowest rate of obesity in modern cultures.

Another popular model that illustrates the extent to which diet influences the health of a popular is the diet of Alaskan Eskimos. These communities that survive in some of the most strenuous climates on the planet and eat a diet that is nearly 70% fats still manage to astonish their western meat-eating counterparts with an extremely low incidence of cardiac illnesses and also joint and skin diseases. Research reveals that their diet includes lot of fish, rich in the two major $\omega$-3 Fatty Acids - Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA).
Chapter 1

Introduction

The role of these essential fatty acids in promoting cardiac health stems from the fact that they can improve the performance of heart and bring down cholesterol levels and blood pressure. And in so doing they protect us against Cardiovascular Diseases (CVD). One cannot overemphasize the import of this in healthcare: according to WHO Fact Sheets CVDs is the number one killer in the world accounting for 29% (17.1 million) deaths globally in 2004. By lowering blood pressure in hypertensive individuals PUFAs can axe almost half of all strokes and ischemic heart diseases. The physical inactivity brought about by our lifestyle and nature of employment is the fourth most threatening health risk as it causes around 21–25% of breast and colon cancer. It is a well known fact that PUFAs can lower the risk of cancer and assist in its treatment. So their presence in our diet can go a long way in eliminating the risk of cancer, and treatments can be augmented by an inclusion of PUFA supplements as well.

PUFAs are also essential for the proper development of brain. A markedly positive influence of these bio-molecules has been noted on neurological disorders like Attention Deficit Hyperactive Disorders in Children, brain disorders like Alzheimer’s and mental illnesses like depression and bipolar disorder. Researchers are also optimistic about the assistance PUFAs can provide in the treatment of diseases like lupus and psoriasis.

PUFAs are absolutely essential to the body in performing its cellular functions effortlessly. Cell to cell signalling and biochemical functions at molecular levels are mediated and perfected by the presence of these fatty acids in our body. They also form precursors of substances with hormonal regulator properties. Bolstering our immune system, both EPA and DHA
help to check abnormal immune responses in asthma, rheumatoid arthritis, kidney inflammation and eczema. Both gestating and lactating mothers and their babies can benefit from an inclusion of these fatty acids in the diet, which helps proper brain development and good vision.

Fish, being high in PUFA content and low in harmful cholesterol, is an ideal source of EPA and DHA for regular consumption. Fishes vary in the abundance of these molecules. If one is hoping to include more omega 3 fatty acids in the diet the best choices are sardines, mackerel, salmon, herring and menhaden. Sardines, are one of the most abundant and cheaply available fishes across the Indian coast line, has long been an integral part of the diet in Kerala, Coastal Karnataka and Tamil Nadu.

With many countries of the Indian Ocean belt – almost 500 million people - face a serious deficiency of proteins. Sardines can be utilized as an affordable source of excellent proteins. Sardine fishery, if put to proper use, and underpinned by spreading awareness, can bring down underweight in children, which is the major global contributor to increasing Disability-Adjusted Life Years (DALY) - the number of years lost due to ill-health, disability or early death. Sardines have now achieved added significance because of the discovery that it is a fish exceptionally rich in EPA and DHA. A regular inclusion of the fish in the diet can contribute a great deal to healthcare and help us grapple more effectively with cardiac illnesses, diabetes and cancer that affect a large segment of our population.

A steady supply of fresh sardines is possible only across our coastal belt. For the benefit of inland populations an effective processing method can be developed which will ensure that they get the valuable components in the
fish intact. This will also guarantee a longer shelf life and perennial availability.

In keeping with the trend of the day, brands of everyday food such as fruit juices, breads, spreads, margarines, snack bars, yogurt, fish products, eggs and children’s beverages enriched with DHA and EPA have invaded market shelves. It is a quick and effective method to deliver the necessary fatty acids to the body, even for strict vegetarians. Trials to produce PUFA-enriched eggs by feeding marine PUFA to egg laying hens have yielded encouraging results. Even fast food like hot dogs and frankfurters can be absolved from the infamy of ‘greasy foods’ by enriching them with PUFA. It helps people to eat healthy even when they are on the move.

**Review of literature**

**1.1 Clupeoids and Sardines**

Sardines are a group of small fishes classified under three genus; *Sardina*, *Sardinops* and *Sardinella*; together consisting of 23 species (Table 1) under the widely categorized group of fishes known as Clupeoids. Clupeoid fishes are small teleosts which are relatively ‘unevolved’ and typically are less than 20 or 30 cm in length; they have no barbules and are frequently laterally compressed, with a series of hard scutes along the ventral surface of the body. Most are soft-bodied, difficult to handle, and covered with rather deciduous scales. Their flesh is often very oily, and the oil content normally varies seasonally, even in low latitude species (Longhurst 1971). These fishes are commercially extremely important – in fact clupeoid fishes like Herrings, Pilchards and Sardines form the main stay of economy of the European maritime nations – as aptly coined by the
famous French expression ‘la crise sardinere’ refering to the disastrous effect of the failed sardine fishery and its impact on the nation. While only a single genus (Clupea) of clupeoids is important in high latitudes and the cold temperate regions, in the warm temperate mid-latitudes there are approximately 10 important genera, dominated by *Sardina, Sardinops, Engraulis, Brevoortia, Etlzmidium and Opistonema*. In low latitudes more than 25 genera occur, many of which are important of which *Sardinella* being the prime (Longhurst 1971).

One of the most significant characteristics of clupeoids from a behavioural and fisheries viewpoint is their occurrence in dense and often very large schools containing many hundreds of thousands of fish which may weigh up to more than 100 tons (Longhurst 1971). Such large schools generally occur in the open ocean, particularly for pelagic sardines and frequently have a diagnostic shape and shoaling behaviour which enabled experienced fishermen to identify them. Clupeiformes also congregate in smaller, less-organized shoals, particularly during spawning seasons. In addition to schooling, some clupeoid fishes may migrate inshore or across latitudes on a seasonal basis (Longhurst 1971).

Most Clupeiformes filter feed by straining water through their long and numerous gill rakers. They consume plankton, particularly small crustaceans and the larval stages of larger crustaceans and fishes. The species which have a diet in which phytoplankton appears to be the preferred component, feed by filtering large diatoms and other phytoplanktons from the water by means of elongated gill rakers which form a filtration sieve. Though few in number, from a fisheries point of
view these species are some of the most interesting, both because of their occurrence in great quantity and also because they represent the most efficient possible utilization of the results of primary production. Indian Oil-sardine *Sardinella longiceps* (Peterson 1956; Nair 1960) fall into this category.

The Indo-Pacific region also contains the greatest number and diversity of species of clupeoids in the tropical oceans; along the mainland of Asia from China to the Red Sea, down the coast of eastern Africa, and through the Indonesian, the Philippine, and the Australian archipelagos. In Indian waters, the clupeoids are chiefly represented by Sardines, Anchovies, White-taits among which Indian Oil Sadrine (*Sadinella longiceps*) ranks as the most valuable and forms the backbone of the fishery of the west coast of India. At least three other species of Sardines can also be found in the southwest coast of India where this study was conducted viz. *Sardinella fimbriata, Sardinella gibbosa, Sardinella jussieu*; listed in decreasing order of abundance. The present study concentrates on two widely available Sardines in this area – *S. longiceps* and *S. fimbriata* and attempts to compare their fatty acid profile with their corresponding variations in bioactivity.
## Sardines of the World

### Table 1: Sardines of the World

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Pilchard</td>
<td><em>Sardina pilchardus</em></td>
<td>NE Atlantic: Iceland (rare) and N. Sea, southward to Bay de Gorée, Senegal. Mediterranean (common in the W. part and in Adriatic Sea, rare in the E. part), Sea of Marmara and Black Sea</td>
</tr>
<tr>
<td>South American Pilchard</td>
<td><em>Sardinops sagax</em></td>
<td>Indo-Pacific: S. Africa to the E. Pacific. Three lineages: S. Africa (<em>ocellatus</em>) and Australia (<em>neopilchardus</em>), Chile (<em>sagax</em>) and California (<em>caeruleus</em>) and Japan (<em>melanostictus</em>)</td>
</tr>
<tr>
<td>Bleeker's blacktip</td>
<td><em>Sardinella atricauda</em></td>
<td>W. Pacific: Indonesia</td>
</tr>
<tr>
<td>Round Sardinella</td>
<td><em>Sardinella aurita</em></td>
<td>E. Atlantic: Gibraltar to Saldanha Bay, S. Africa. Also known from the Mediterranean and Black Sea. W. Atlantic: Cape Cod, USA to Argentina. Bahamas, Antilles, Gulf of Mexico and Caribbean coast</td>
</tr>
<tr>
<td>Deepbody Sardinella</td>
<td><em>Sardinella brachysoma</em></td>
<td>Indo-West Pacific: Madagascar (but apparently not elsewhere in the W. Indian Ocean), Madras, Indonesia, N. Australia</td>
</tr>
<tr>
<td>Fiji Sardinella</td>
<td><em>Sardinella fijiense</em></td>
<td>W. Pacific: Papua New Guinea and Fiji. Reported from New Caledonia</td>
</tr>
<tr>
<td>Fringescale Sardinella</td>
<td><em>Sardinella fimbriata</em></td>
<td>Indo-West Pacific: S. India and Bay of Bengal to the Philippines, also E. tip of Papua New Guinea</td>
</tr>
<tr>
<td>Goldstripe Sardinella</td>
<td><em>Sardinella gibbosa</em></td>
<td>Indo-West Pacific: Persian Gulf, East Africa and Madagascar to Indonesia, north to Taiwan and Korea south to the Arafura Sea and northern Australia.</td>
</tr>
<tr>
<td>Taiwan Sardinella</td>
<td><em>Sardinella hualiensis</em></td>
<td>NW Pacific: Taiwan, possibly S. to Hong Kong</td>
</tr>
<tr>
<td>Brazilian Sardinella</td>
<td><em>Sardinella janeiro</em></td>
<td>W. Atlantic: Gulf of Mexico, Caribbean, W. Indies S. to Brazil and N. Uruguay</td>
</tr>
<tr>
<td>Mauritian Sardinella</td>
<td><em>Sardinella jussieu</em></td>
<td>W. Indian Ocean: W. coasts of S. India, from Bombay S. to Sri Lanka; also to Madagascar and Mauritius. NW Pacific: Taiwan, Hong Kong and Viet Nam</td>
</tr>
</tbody>
</table>

Bioactivity Profile of Polysaturated Fatty acid extracts from *Sardinella longiceps & Sardinella fimbriata* A Comparative Study
<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bali Sardinella</strong></td>
<td><em>Sardinella lemuru</em> E. Indian Ocean: Phuket, Thailand; southern coasts of E. Java and Bali; and W. Australia. W. Pacific: Java Sea, Philippines, Hong Kong, Taiwan Island, S. Japan</td>
</tr>
<tr>
<td><strong>Indian Oil Sardine</strong></td>
<td><em>Sardinella longiceps</em> Indian Ocean: N. and W. parts only, Gulf of Aden, Gulf of Oman, but apparently not Red Sea or the Persian Gulf, E. to S. part of India, on E. coast to Andhra; possibly to the Andaman Islands.</td>
</tr>
<tr>
<td><strong>Madeiran Sardinella</strong></td>
<td><em>Sardinella maderensis</em> E. Atlantic: Gibraltar to Angola; single specimen recorded from Walvis Bay, Namibia. Also known from the Mediterranean (S. and E. parts, also penetrating Suez Canal).</td>
</tr>
<tr>
<td><strong>Marquesan Sardinella</strong></td>
<td><em>Sardinella marquesensis</em> E. Pacific: endemic to the Marquesan Islands. Introduced into Hawaiian waters.</td>
</tr>
<tr>
<td><strong>Blacktip Sardinella</strong></td>
<td><em>Sardinella melanura</em> Indo-West Pacific: Gulf of Aden S. to Madagascar and Mauritius and E. to the Arabian Sea and NW India (apparently not found S. of Bombay nor in N. Bay of Bengal); then from Indonesia (but not in S. China Sea) to Samoa. Reported from the Penghu Islands</td>
</tr>
<tr>
<td><strong>East African Sardinella</strong></td>
<td><em>Sardinella neglecta</em> W. Indian Ocean: Somalia, Kenya, and Tanzania</td>
</tr>
<tr>
<td><strong>Richardson's Sardinella</strong></td>
<td><em>Sardinella richardsoni</em> NW Pacific: Hainan Island, Hong Kong, China.</td>
</tr>
<tr>
<td><strong>Yellowtail Sardinella</strong></td>
<td><em>Sardinella rouxi</em> E. Atlantic: Senegal to Congo and perhaps S. of Angola.</td>
</tr>
<tr>
<td><strong>Sind Sardinella</strong></td>
<td><em>Sardinella sindensis</em> W. Indian Ocean: Arabian Sea, from Gulf of Aden to the Persian Gulf and Bombay</td>
</tr>
<tr>
<td><strong>Freshwater Sardinella</strong></td>
<td><em>Sardinella tawilis</em> Endemic to Lake Taal (= Lake Bombon), Luzon, Philippines</td>
</tr>
<tr>
<td><strong>Japanese Sardinella</strong></td>
<td><em>Sardinella zunasi</em> W. Pacific: S. coasts of Japan S. to about Taiwan</td>
</tr>
</tbody>
</table>

**Source:** [www.fishbase.org](http://www.fishbase.org)
1.1.1 Taxonomy

Taxonomic positions of the two test species, *Sardinella longiceps* and *Sardinella fimbriata* are given below

Kingdom Animalia

- Phylum Chordata
- Class Actinopterygii
- Order Clupeiformes
- Family Clupeidae
- Genus Sardinella
  - *Sardinella longiceps* (Valenciennes, 1847)
  - *Sardinella fimbriata* (Valenciennes, 1847)
Chapter 1

Introduction

*Sardinella longiceps* is identified by its sub-cylindrical elongated body with its ventral profile evenly convex. It can be conclusively separated from all other clupeids in the northern Indian Ocean by its longer head and lower gill rakers. Caudal fin is well forked, lobes pointed; two large alar scales can be seen at the base, colour bluish green back with golden reflections, abdomen silvery with pinkish tinge and a faint golden spot behind gill opening are other in-hand diagnosis (Whitehead 1985).

*Sardinella fimbriata* can be identified by its compressed, flattened body and conclusively by its total number of scutes which varies consistently from 29 to 33. Vertical striae on scales do not meet at center, hind part of scales have a few perforations and somewhat produced posteriorly. A dark spot at dorsal fin origin also can be seen (Whitehead 1985).

*Sardinella longiceps* is an extremely valuable commercial fish and is also the most important clupeoid fishery of the whole of western Indo-Pacific. Stocks of this species extend around the whole perimeter of the northern part of the Indian Ocean from the Gulf of Aden to the Bay of Bengal and also occur in the Indonesian archipelago and the Philippines. Malabar region is considered as the zone of maximum abundance. The landings of this important species in India have reached as much as 200 thousand tons per annum in some years (Mohanty et al.). During the Second World War, people of Kerala purely sustained themselves on the traditional dish of sardine and tapioca to save them from the bitter famine. However, the fishery is susceptible to irregular and large-scale fluctuations in resource availability and hence it has been studied intensively by fishery
biologists in India, beginning in the early 1920s with the work of Hornell and his associates. When Central Marine Fisheries Research Institute (CMFRI) was established in 1947, its top priority area was to study the Indian Oil Sardine. Since then, investigations on systematics, fishery, food and feeding, growth, distribution, reproduction, nutritional value and processing were extensively carried out which enriched our knowledge on this species. The shoals of Indian Oil Sardines become available to the fishery towards the end of June, when populations of adults with mature gonads appear near the coast and progressively move northwards as the season advances; these fish have mature gonads and spawn during their first few months in the coastal region. As the season advances, a second wave of shoals arrives in the coastal region and becomes available to the fishery; these are younger, immature fish and their availability reaches a peak during the months of October to December. Approximately the same cycle of events is repeated annually on the east coast of India. The arrival of the first wave of adult sardines at the coast generally coincides with the onset of the southwest monsoon; at this time there is a very strong seasonal bloom of phytoplankton, principally of the diatom *Fragillaria sp.* and it is supposed that the spawning migration is timed to coincide with this; the arrival of the second wave of such shoals, at a peak in October to December coincides with a second phytoplankton bloom. Nair (1960) has shown that the stomach contents of this species are dominated by phytoplankton, largely a single species of the diatom (*Fragillaria oceanica*) and dinoflagellates. He has suggested that the large and important fluctuations in the availability of the species from year to year may be dependent upon the nature and timing of the annual bloom of this diatom.
S. fimбриата is comparatively a less important lesser Sardine in terms of the trade and the species has an average annual landing of around 50000 tonnes in India (Mohanty et al.). This species is found in the local fisheries of Philippines, in the Visayan Sea in Indonesia, along the south-east coast of Bay of Bengal and southern coast of the Arabian Sea, and along the north coast of the Australian continent. This species occurs in commercially significant quantities in the southern part of Indian coast. These are mostly zooplankton-feeders and where their diet has been investigated (Ronquillo 1960) it is evident that they subsist upon a mixed diet, dominated by crustacea of various sorts, according to the relative availability from place to place and season to season. Spawning season extends from August to February (Bennet 1965) with juveniles appearing in the catch almost at the same time as S. longiceps.

1.2 Marine Lipids and Polyunsaturated Fattyacids

Marine lipids from sardines come under two categories of fattyacids; Saturated Fattyacids (SFA) and Unsaturated Fattyacids (USFA). Unsaturated Fattyacids are characterized by the presence of at least one double bond between the carbons. Unsaturated fattyacids consist of monounsaturated fattyacids (MUFA) and polyunsaturated fattyacids (PUFA). There are two classes of PUFAs, ω3 and ω6. The distinction between ω3 and ω6 fattyacids is based on the location of the first double bond, counting from the methyl end of the fattyacid molecule. ω3 and ω6 fattyacids are also known as essential fattyacids (EFAs) because humans, like all mammals, cannot make them and must obtain them in their diet (Bendich and Deckelbaum 2005).

Bioactivity Profile of Polyunsaturated Fatty acid extracts from Sardinella longiceps & Sardinella fimбриата: A Comparative Study
Chapter 1

Introduction

The base origin of most marine lipids are from phytoplankton which are rich in PUFA (Klein Breteler et al. 1999). However, zooplanktons are also known to assimilate PUFA with higher levels of unsaturation. This extra level of unsaturation is partly achieved in zooplankton from their microplankton based diet. Microplanktons are known to preferentially increase the level of unsaturation in the food chain by converting the PUFA obtained from phytoplankton to higher degrees of unsaturation (Kleppel et al. 1998, Klein Breteler et al. 1999). Two important naturally occurring ω3 fatty acids that are entirely marine based, are Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) (Klein Breteler et al. 1999). EPA and DHA are essential as structural components of all the cell walls. They are necessary for proper brain and eye development, and are required for the proper functioning of the immune, reproductive, respiratory and circulatory systems (Simopoulos 1991).

1.2.1 Eicosapentaenoic Acid (EPA)

EPA, systematically called all-cis-5, 8, 11, 14, 17-icosapentaenoic acid (Figure 1), is a carboxylic acid with a 20-carbon chain with five cis-double bonds (sometimes denoted as C20:5(n-3)). This FA is involved in the production of eicosanoids, which are hormone-like substances which act as vasodilators and anti-platelet aggregators. EPA is a precursor to the eicosanoids known as series 3 prostaglandins and thromboxanes and series 5 leukotrienes (Arthur 1999).

![EPA Structure](image)

Figure 1: EPA Structure
1.2.2 Docosahexaenoic acid (DHA)

DHA, systematically called as \textit{all-cis}-docosa-4,7,10,13,16,19-hexaenoic acid (Figure 2), is a carboxylic acid with a 22-carbon chain and six cis double bonds (denoted sometimes as C22:6\textit{n-3}). DHA is an important component of our brain and eyes. It is fundamentally important in the neurological growth and development of children, and for their eyesight (Arthur 1999).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{DHAStructure.png}
\caption{DHA Structure}
\end{figure}

1.2.3 Metabolism

Omega 6 fatty acids are represented by linoleic acid (LA) and the corresponding \textomega 3 fatty acids by \textomega 6-linolenic acid (ALA). LA is plentiful in nature and is found in the seeds of most plants except for coconut, cocoa, and palm. ALA on the other hand is found in the chloroplast of green leafy vegetables. Both EFAs can be metabolized to longer-chain fatty acids of 20 and 22 carbon atoms. LA is metabolized to arachidonic acid (AA) and ALA, to EPA and DHA, increasing the chain length and degree of unsaturation by adding extra double bonds to the carboxyl group. Humans and animals except carnivores such as lions and cats can convert LA to AA and ALA to EPA and DHA (de Gomez & Brenner 1975). There is competition between \textomega 3 and \textomega 6 fatty acids for the desaturation enzymes. However, both 1-4 and 1-6 desaturase prefer \textomega 3 to \textomega 6 fatty acids. There is some evidence that 1-6 desaturase decrease with age. Premature infants, hypertensive individuals,
and some diabetics are limited in their ability to make EPA and DHA from ALA. These findings are important and the role of fish oils which are natural sources of EPA and DHA is quite significant in these cases (Simopoulos 1991). AA is found predominantly in the phospholipids of grain-fed animals. LA, ALA, and their long-chain derivatives are important components of animal and plant cell membranes. In mammals and birds the ω3 fatty acids are distributed selectively among lipid classes. ALA is found in triglycerides, in cholesteryl esters, and in very small amounts in phospholipids. EPA is found in cholesteryl esters, triglycerides, and phospholipids. DHA is found mostly in phospholipids. In mammals, including humans, the cerebral cortex, retina, and testis and sperm are particularly rich in DHA. DHA is one of the most abundant components of the brain’s structural lipids. DHA, like EPA, can be derived only from direct ingestion or by synthesis from dietary EPA or ALA (Simopoulos 1991).

It has been reported that conversion of ALA to EPA and further to DHA in humans is limited, but varies with individuals. Women have higher ALA conversion efficiency than men, probably due to the lower rate of utilization of dietary ALA for beta-oxidation. This suggests that biological engineering of ALA conversion efficiency is possible (Hussein et al. 2005). Goyens et al. (2006) suggest that it is the absolute amount of ALA, rather than the ratio of $n-3$ and $n-6$ fatty acids, which affects the conversion. However, ALA-feeding studies and stable-isotope studies using ALA, which have addressed the question of bioconversion of ALA to EPA and DHA, have concluded that in adult men conversion to EPA is limited (approximately 8%) and conversion to DHA is extremely low (<0.1%). In women fractional conversion to DHA appears to be greater (9%), which
may partly be a result of a lower rate of utilisation of ALA for beta-oxidation in women. In this context, direct intake of sufficient quantities of these ω3 FA is essential for the stable metabolism of the body (Simopoulos 1991).

### 1.3 Sardines and PUFA

Clupeid fishes are known to be seasonal feeders. They store great reserves of energy for maintenance during the times when food is scarce. Fishes store their energy as lipids and these compounds are burnt when energy has to be expended. They are also useful in maintaining stability, permeability and fluidity of the cell membranes. Fat reserves and fattyacid composition of the fishes can vary with age, sex and season. This has been proved in several species of clupeids including *S. longiceps* (Gopakumar 1965) apart from other *Sardinops* (Gamez-Mezza *et al.* 1999, Shirai *et al.* 2002) and *Sardina* (Bandarra *et al.* 1997).

Easy availability of these species in the western coast in great quantities roughly all through out the year means a ready availability of enormous amounts of these essential FAs for human consumption. This brings in a huge commercial implication for the fishery industry as this can potentially supplement a viable nutritional and pharmaceutical industry solely based on Marine PUFA.

### 1.4 PUFA and Nutrition

Scientists were first alerted to the many benefits of fish oils in the early 1970s when Danish physicians observed that Greenland Eskimos had an exceptionally low incidence of heart disease and arthritis despite the fact
that they consumed a high-fat diet. Intensive research soon discovered the secret that two of the fats (oils) they consumed in large quantities, EPA and DHA, were actually highly beneficial. More recent research has established that fish oils (EPA and DHA) play a crucial role in the prevention of atherosclerosis, heart attack, depression, and cancer.

Seemingly minor differences in their molecular structure make the two EFA families act very differently in the body. While the metabolic products of \( \omega_6 \) FA promote inflammation, blood clotting, and tumor growth, the \( \omega_3 \) FA act entirely opposite (Caygill et al. 1996). Although both \( \omega_3 \)s and \( \omega_6 \)s are needed, it is becoming increasingly clear that an excess of \( \omega_6 \) FA can have dire consequences. Many scientists believe that a major reason for the high incidence of heart disease, hypertension, diabetes, obesity, premature aging, and some forms of cancer is the profound imbalance between our intake of \( \omega_6 \) and \( \omega_3 \) FAs. Our ancestors evolved on a diet with a ratio of \( \omega_6 \) to \( \omega_3 \) of about 1:1. A massive change in dietary habits over the last few centuries has changed this ratio to something closer to 20:1 and this spells trouble (Simopoulos 1991).

Recognizing the unique benefits of EPA and DHA and the serious consequences of a deficiency the US National Institutes of Health recently published Recommended Daily Intakes of fattyacids. It recommends a total daily intake of 650 mg of EPA and DHA, 2.22 g/day of ALA and 4.44 g/day of LA. Saturated fat intake should not exceed 8% of total calorie intake or about 18 g/day.
1.4.1 Pregnancy & Child Birth

An adequate intake of DHA and EPA is particularly important during pregnancy and lactation. During this time the mother must supply all the baby's needs because it is unable to synthesize these essential fatty acids itself. DHA makes up 15 to 20% of the cerebral cortex and 30 to 60% of the retina (Gal et al. 2005). There is some evidence that an insufficient intake of ω3 fatty acids may increase the risk of premature birth and an abnormally low birth weight (Carlson 1999, Cunnane et al. 2000, Makrides et al. 2000). There is also emerging evidence that low levels of omega-3 acids are associated with hyperactivity in children (Mitchel et al. 1987). The constant drain on a mother's DHA reserves can easily lead to a deficiency and it is believed that pre-eclampsia and postpartum depression could be linked to a DHA deficiency. Experts recommend that women get at least 500-600 mg of DHA every day during pregnancy and lactation (Carlson 1999).

1.4.2 Depression

The human brain is one of the largest "consumers" of DHA. A normal adult human brain contains more than 20 grams of DHA. Low DHA levels have been linked to low brain serotonin levels which again are connected to an increased tendency to depression, suicide, and violence (Edwards et al. 1998). Studies have shown that countries with a high level of fish consumption have fewer cases of depression (Hibbeln 1998). Researchers at Harvard Medical School have successfully used fish oil supplementation to treat bipolar disorder (Stoll et al. 1999) and British researchers report encouraging results in the treatment of schizophrenia (Laugharne et al. 1996).
1.4.3 Cardiac Disorders

Danish researchers have concluded that fish oil supplementation may help prevent arrhythmias and sudden cardiac death in healthy men (Christensen et al. 1999). An Italian study of 11,000 heart attack survivors found that patients supplementing with fish oils markedly reduced their risk of another heart attack, a stroke or death. A group of German researchers found that fish oil supplementation for two years caused regression of atherosclerotic deposits (von Schacky et al. 1999) and American medical researchers report that men who consume fish once or more every week have a 50% lower risk of dying from a sudden cardiac event than do men who eat fish less than once a month (Siscovick et al. 1995). Fish oil supplementation (10 grams/day) reduces the number of attacks by 41% in men suffering from angina (Salachas et al. 1994). It is found that fish oil supplementation reduces the severity of a heart attack and supplementation started immediately after a heart attack reduces future complications (Eritslund et al. 1994). Bypass surgery and angioplasty patients reportedly also benefit from fish oils and clinical trials have shown that fish oils are beneficial for heart disease patients (Singh et al. 1997). Fish oils are especially important for diabetics who have an increased risk of heart disease. It is found that supplementing with as little as 2 grams/day of fish oil (410 mg of EPA plus 285 mg of DHA) can lower diastolic pressure by 4.4 mm Hg and systolic pressure by 6.5 mm Hg in people with elevated blood pressure (Appel et al. 1993).
1.4.4 Rheumatic Disorders

Fish oils are particularly effective in reducing inflammation and can be of great benefit to people suffering from rheumatoid arthritis or ulcerative colitis. Daily supplementation with as little as 2.7 grams of EPA and 1.8 grams of DHA can markedly reduce the number of tender joints and increase the time before fatigue sets in (Kremer 2000). Some studies have also noted a decrease in morning stiffness (Fortin et al. 1995) and clinical trials concluded that arthritis patients who took fish oils could eliminate or sharply reduce their use of NSAIDs and other arthritis drugs (Kremer et al. 1995).

1.4.5 Cancer

There is also considerable evidence that fish oil consumption can reduce the risk of breast and prostate cancer (Chavarro et al. 2008) and help slow their progression (Caygill et al. 1996). Daily supplementation with fish oils has been found effective in preventing the development of colon cancer (Mehta et al. 2008). There is now also considerable evidence that fish oil consumption can delay or reduce tumor development in breast cancer. Studies have also shown that a high blood level of omega-3 fatty acids combined with a low level of ω6 acids reduces the risk of developing breast cancer (Soto-Guzman et al. 2010). Daily supplementation with as little as 2.5 grams of fish oils has been found effective in preventing the progression from benign polyps to colon cancer (Fernandez-Banares et al. 1996) and Korean researchers reported that prostate cancer patients have low blood levels of omega-3 fatty acids (Yang et al. 1999). Greek researchers report
that fish oil supplementation improves survival and quality of life in terminally ill cancer patients (Gogos 1998).

1.4.6 Omega 3 Enriched Products

Omega 3 fatty acids are being increasingly promoted as important dietary components for health and disease prevention. These fatty acids are naturally enriched in fatty fish like salmon and tuna and in fish-oil supplements. An increasing number of foods that are not traditional sources of ω3 fatty acids, such as dairy and bakery products, are now being fortified with small amounts of these fatty acids (Surette 2008).

In conclusion, the direct and indirect nutritional advantages of ω3 fatty acids have been recognised by the medical community and increasing presence of ω3 enriched food products is a testimony to this fact.

1.5 PUFA and Pharmaceuticals

Omega 3 oils, though called ‘miracle food’ of the 21st century, are not a ‘miracle drug’ in itself. Its use in pharmaceutical industry is always in combination with a more direct drug and the presence of these FA induce a favourable condition in the patient’s body for the real drug to be effective.

FA in combination with drugs for the treatment of diseases is an area of immense interest because it opens a new field in pharmaceutical research - ω3 fatty acids in the control of metabolic and autoimmune disorders, that includes CVD, arthritis, nephrites, psoriasis, ulcerative colitis and cancer (Simopoulos et al. 1991). Preliminary data from animal and human studies suggest that the concurrent ingestion or administration of ω3 fatty acids with
drugs leads to potentiation of drug effects, as with propranolol, which may lead to a decrease both in the dose of ω3 fatty acids and in the drug dose or, as with cyclosporine, to a decrease in toxicity of the drug. By partially replacing the fatty acids of phospholipids in the cell membranes, ω3 fatty acids modify enzymes, receptors, and other proteins (Simopoulos et al. 1991). Additional studies suggest that the incorporation of ω3 fatty acids by cell membranes is enhanced in the presence of olive oil and linseed oil, emphasizing once again the importance of nutrient interactions (Cleland et al. 1991). Cyclosporin is used widely in organ transplantation and in many individuals its use leads to impairments in renal function and increased thromboxane formation. It was noted that the use of fish oil instead of olive oil as the vehicle for its administration in rats led to attenuation of the cyclosporine nephrotoxicity (Elzinga et al. 1987) without affecting thromboxane synthesis (Walker et al. 1989).

There is much scope for research in finding out new combinations of drugs with ω3 FA and delving deep into the causes for the interactions that happen in the body. Research in these aspects is still in budding stage. Fishery biology, fishing techniques, taxonomy, size distribution, nutrient value and processing of Sardines have been extensively worked out in research institutions like Centre Marine Fisheries Research Institute and Central Institute of Fisheries Technology. However, there have been hardly any investigations relating to the industrial applications of this fish commodity despite being cheap and available round the year. Hence, the present work attempts to fill this gap.
1.6 Research Objectives

The present study revolves primarily around 3 specific intentions.

a) Explicate the importance of Sardines found in the west coast of India.

b) Examine the preferential bioactivity of EPA and DHA.

c) Evaluate the pharmaceutical applications of marine PUFA from widely available sources.

As the fattyacid profile of sardines is expected to change across seasons, the first target was to analyze the seasonal change in all kinds of fatty acids for the two study species. As per those results, a good extraction technique was to be standardized to obtain a substantially pure polyunsaturated fattyacid extract which can be suggested for use in clinical research. Based on these objectives, the study is crystallized into following chapters.

Chapter 2 deals with the seasonal variation of fattyacids in S longiceps and S fimbriata, with emphasis on how EPA and DHA varies across seasons in the two species of sardines. An explanation is attempted to elucidate why the quantity of these fatty acids vary across seasons in relation to their feeding habits.

Chapter 3 elaborates the extraction procedure of polyunsaturated fattyacids from S longiceps and S fimbriata and compares on how this particular procedure fares with other known procedures for extracting polyunsaturated fatty acids.
Chapter 4 deals with the activity of the polyunsaturated fatty acid extracts against a selected set of gram positive and gram negative bacteria. Results obtained are analysed in the light of contemporary research and compared to evaluate the validity of the findings.

Chapter 5 elaborates an in vivo study on how this polyunsaturated fatty acid extracts influence the levels of various biochemical parameters in diabetes induced mice tested against controls. Recovery profiles for both species for different biochemical parameters are compared and results explained relating them with contemporary research findings.

Chapter 6 details the cytotoxic activity of the polyunsaturated fatty acid extracts on two cancer cell lines, MCF-7 and DU-145. Differences in cytotoxic activity between the species is explained based on their PUFA profiles.

Chapter 7 summarizes the present study with recommendations for a sardine based pharmaceutical/nutritional venture.

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Bioactivity Profile of Polyunsaturated Fatty acid extracts from Sardinella longiceps & Sardinella finbriata A Comparative Study