Introduction

From times immemorial, desire for consumption of animal products for good health or for pleasing the palate has existed. Fish, a staple diet in many parts of India, is known for its high nutritive value, taste, and easy digestibility. It provides high amounts of proteins (17-20%) and a wide variety of minerals, including vitamins A and D, phosphorus, magnesium, selenium, and iodine. Its protein—like that of meat—is easily digestible and favorably complements other dietary proteins. Asia, which combines a relatively high per capita consumption with a large population, is by far the most important fish-consuming region in the world. About 75% of the world fish catch is used for human consumption. In recent years, the volume of fishery products marketed in their fresh state has increased, as has that of frozen fish (FAO, United Nations, 1996).

Fish is a good source of all essential amino acids. Fish proteins are rich in lysine and threonine, thus effectively supplementing cereal proteins. Small fish that are eaten along with bones are also a rich source of calcium. The most important reason why fish is currently so popular is its omega-3 fatty acids which are essential for the healthy development of the eyes, brain, and proven studies showing that eating fish decreases heart diseases (Fishery Information, Data and Statistics unit, FIDI, 2002). The caloric value of the fish flesh depends upon its fat content and thus the season. During the spawning season, the fat content may rise with increase in the caloric value. Fat (13-15%) and moisture (64%) in fish are the most variable constituents depending on the season as well as species when compared to other flesh foods.

Aquaculture or farming of fish, shrimp, shellfish, and seaweeds has existed for nearly 4,000 years, especially in Asia. Unprecedented growth in aquaculture production in the last decade has given it increased importance in the modern food industry. Virtually all farmed fish is used for human consumption, while one third of the conventional fish catch is used to make fish meal and fish oil. Today, one fourth of the fish consumed by
humans is the product of aquaculture and this percentage will increase as aquaculture expands and the world’s conventional fish catch from oceans and lakes continues to decline due to over fishing and environmental damage (FAO, 2002).

Asia dominates world aquaculture, producing four fifths of all farmed fish, shrimp, and shellfish. China is by far the leading producer, contributing nearly 69.6 percent of the total quantity. Aquaculture accounts for more than half of China’s total fish production with India as the second largest producer, with 6.3 percent of the world’s total aquaculture (Table. 1.1).

Table 1.1 Top ten producers in aquaculture production: quantity

<table>
<thead>
<tr>
<th>Producer</th>
<th>2002</th>
<th>2004</th>
<th>APR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(in 1,000 tonnes)</td>
<td>(in %)</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>27 767 251</td>
<td>30 614 968</td>
<td>5.0</td>
</tr>
<tr>
<td>India</td>
<td>2 187 891</td>
<td>2 472 335</td>
<td>6.3</td>
</tr>
<tr>
<td>Vietnam</td>
<td>703 041</td>
<td>1 198 617</td>
<td>30.6</td>
</tr>
<tr>
<td>Thailand</td>
<td>954 567</td>
<td>1 172 866</td>
<td>10.8</td>
</tr>
<tr>
<td>Indonesia</td>
<td>914 071</td>
<td>1 045 051</td>
<td>6.9</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>786 604</td>
<td>914 752</td>
<td>7.8</td>
</tr>
<tr>
<td>Japan</td>
<td>826 715</td>
<td>776 421</td>
<td>-3.1</td>
</tr>
<tr>
<td>Chile</td>
<td>545 655</td>
<td>674 979</td>
<td>11.2</td>
</tr>
<tr>
<td>Norway</td>
<td>550 209</td>
<td>637 993</td>
<td>7.7</td>
</tr>
<tr>
<td>United States of America</td>
<td>497 346</td>
<td>606 549</td>
<td>10.4</td>
</tr>
<tr>
<td>Top ten subtotal</td>
<td>35 732 648</td>
<td>40 114 531</td>
<td>6.0</td>
</tr>
<tr>
<td>Rest of the world</td>
<td>4 650 830</td>
<td>5 353 825</td>
<td>7.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>40 383 478</td>
<td>45 468 356</td>
<td>6.1</td>
</tr>
</tbody>
</table>

*Source:* (FAO, Rome. 2006) APR- Annual production rate
Aquaculture products fall into two distinct groups: high-value species such as shrimp and salmon that are mostly grown for export, and low-value species such as carp and tilapia that are consumed primarily locally. China, for instance, raises a substantial amount of shrimp in intensively managed ponds along its coastline for the lucrative export trade (Biksham and Andrea, 1996).

Fish plays an important role in the nutrition of people in India. Per capita availability of fish in the country is 9.5 kg with production at 5.8 million tonnes during 1999-2000. Indian aquaculture includes different species viz. Indian major carps (Catla, *Catla catla*; Rohu, *Labeo rohita*; Mrigal, *Cirrhinus mrigala*; and Calbasu, *Labeo calbasu*), marine shrimp, freshwater shrimp, oysters, green and brown mussels, and pearl oysters for domestic and international markets (Gopakumar, 2003).

Fish, though high in its nutritional source has some disadvantages that include spoilage. Spoilage is any change that makes food unfit for consumption and includes chemical and physical changes, such as bruising, browning, infestation by insects or growth of microorganisms such as bacteria, yeasts and molds. High moisture foods like vegetables, fruits, milk, fish, and meat are highly susceptible to microbial spoilage, while low moisture foods such as grains and spices are more stable as they have low moisture content. Food processing and preservation, pH, nutrient content, moisture availability or water activity, O-R potential and presence of inhibitory substances are factors that influence the spoilage or help in stability of a food product. Of all the flesh foods, fish is the most susceptible to autolysis, oxidation and hydrolysis of fat and microbial spoilage.

Being one of the most perishable of foods, fish spoilage is influenced by the species of fish, catching methods, season, nature and acquired microflora, fishing ground, temperature and handling. Fish spoilage is mainly brought about by bacterial, enzymatic, chemical and physical agents. While chemical
and physical spoilage of fish play lesser roles in spoilage, bacterial or microbial spoilage is the most prevalent form of fish spoilage brought about by resident as well as acquired microflora during harvest/ capture, handling and storage along with enzymatic changes.

Biochemical spoilage includes proteolysis, oxidation and increase in pH levels, which are also assisted by the load of bacterial contamination on the skin, gills and viscera. Microbiologically, the surface load and natural microflora aid in the spoilage of the fish if proper processing or preservation steps are not taken.

With increasing demand for fresh fish, the condition under which it is harvested and brought to the port and marketed needs to receive greater attention. In less developed areas, due to lack of adequate refrigeration and transportation facilities most of the seasonal fish landings are sold in the local markets and the rest are cured or dried by traditional methods. For marketing fish in the fresh state, its characteristic texture, flavor, odour and appearance have to be retained. To retain the quality of fish meant for frozen storage or processing, latent changes like biochemical and bacterial should be prevented.

During processing and preservation, the nutritive value of any food should be protected. Muscle tissue composition varies with many factors such as sex, age, maturity and season. Fatty fish show large-scale seasonal variations in lipid, based largely on their reproductive cycle.

Enzyme mediated changes in fish muscle contribute to its quality as also its deterioration. An important biochemical activity in muscle tissue postmortem is reduction in pH due to glycolysis which is inhibited or ceases by action of enzyme phosphofructokinase at low pH of 5.1-5.5 termed as ultimate pH. Ultimate pH is important as it has impact on the textural quality of meat,
Introduction

its water holding capacity, its resistance to growth of microorganisms and its colour. In red meat fishes like mackerel and tuna the ultimate pH is around 5.5 and in lean white fish it is 6.2-6.6 (Fennema, 1985). A rapid drop in the pH of the carcass after harvesting is important to reduce microbial spoilage. Setting of rigor mortis is especially important in preservation of fish for it retards post mortem autolysis and microbial decomposition (Frazier, Food Microbiology). All fish and shellfish in comparison to meat have a much shorter period of rigor mortis and resolution and hence undergo spoilage quickly. Temperature also plays an important role during the onset of rigor.

To preserve fish from undergoing spoilage, certain food preservation techniques help to eliminate conditions that are favorable for the growth of microorganisms. They also destroy certain enzymes, which are responsible for the undesirable chemical and physical changes that naturally occur after harvesting.

Though people knew about preservation methods to prevent food spoilage for centuries, it was the French chemist Louis Pasteur (1857) who demonstrated the role of microorganisms in the process. Food preservation was synonymous with processing in the early days and continues to be so to a large extent even today. Fish preservation techniques developed from quiet an early time because of its extreme perishability. The preservation of seafood by chilling or on ice was practiced as early as 1838 onboard New England trawlers. Besides chilling with ice or brine, traditional methods like drying have evolved with more modern methods of freezing, dehydration, canning, pickling etc. for preservation of fish over the years.

Although these methods have been used for preservation of fish with various degrees of effectiveness, each method also has some drawbacks like the alteration of taste, texture of fresh fish, requirement of cold chains, addition of one or more additives etc. In recent years, consumer resistance to highly
Introduction

processed foods as well as the high cost of intensive processing has led to the
development of minimally processed, cost effective products that are as close
to the fresh condition of the food as possible.

With the advent of modern refrigeration equipment, food preservation
by freezing / chilling has become popular. Low temperature storage slows
down many of the enzymatic reactions involved in spoilage and reduces the
growth rate of microorganisms at storage temperature of 0-4°C. Freezing
allows food to be stored for longer duration than refrigeration and results in the
least physical or chemical changes. Refrigeration, though a shorter-term
preservation than freezing, is sometimes preferred because it avoids textural
changes in fish and has lower associated energy costs. Refrigerated foods
also retain the texture and flavor of the food closest to its original taste.
Worldwide consumer preference for fresh foods not only boosted the
refrigerated foods segment, but also led to its refinement in the form of
vacuum and modified atmosphere packaging. The former is popularly called
as Sous - vide products.

Vacuum packed and refrigerated foods enjoy an extended shelf life
beyond ordinary refrigerated foods as the lack of access to oxygen arrests
oxidative chemical changes and aerobic microbial growth. Modified
atmosphere storage too restricts access to oxygen by replacing air with
oxygen deficient gas mixtures to realize similar benefits. But the anaerobic
nature of these methods also increases the possibility of the growth of the
dangerous pathogen C. botulinum.

The diversity and quantities of vacuum packed and vacuum cooked
prepared meals and menu components are rapidly growing in the European
and American markets. These involve minimal heat processing, high water
activity, absence of preservatives, and the use of many different and often
exotic ingredients, which also have a high-risk potential. Quality is the primary
Introduction

Concern for "sous vide" products and safety must be guaranteed by the application of the 'Hazard Analysis Critical Control Point' (HACCP) concept, along with an adequate combination of different inhibitory factors (degree of heating and cooling, pH, incorporation of starter cultures, bacteriocins and some particular enzymes) (Martens, 1995). Sous vide, also known as cuisine en papillote sous vide, is an interrupted catering system in which raw or par-cooked food is sealed in to a vacuumised laminated plastic pouch or container, heat treated by controlled cooking, rapidly chilled and then reheated for service after a period of chilled storage. The chilled storage period is up to 21 days at 0 to 3°C. The recommended thermal process for sous vide products is 90°C for 10 min, or its time-temperature equivalent (Leadbetter and Sara, 1989).

Consumer demand for foods, which retain their natural flavour, colour and texture but contain very few additives lead to the development of minimal processing technologies. Here the preservation is designed to limit the impact of processing without much change in the nutritional and sensory characters of the food product. To give consumers a finally portioned fish product which is ready to cook, it is necessary to develop a hurdle method of preservation, avoiding if possible the use of modified atmosphere which under a lax storage temperature can become a source of dangerous C. botulinum.

The preservation of almost all foods is based on application of several preservative methods (e.g., heating, chilling, drying, curing, acidification, oxygen-removal, fermenting, adding preservatives, etc.). These methods and their underlying principles (i.e., F, t, a_w, pH, E_h, competitive flora, preservatives, etc.) have been applied empirically since long, but are recently being applied intelligently using the concept of hurdle technology (Leistner, 1992). Hurdle technology involves application of several processing / preservation methods in small amounts that individually are insufficient for preservation, but when combined are sufficient to preserve the food for
reasonably long periods. In developing countries, application of intelligent hurdle technology has proven useful for novel foods, which in spite of minimal processing makes them ambient stable. It is also useful in modifications of traditional intermediate to high-moisture foods.

While there is much interest in minimally processed convenience foods, there are hardly any processed fish products, which fall in this category. Dressed and portioned fish, that are convenient to cook and can be stored under refrigeration for long periods are mostly processed as modified atmosphere products. Modified atmosphere and vacuum packed fish, unless stored strictly at 0°C are risk prone due to possible growth of highly toxic *C. botulinum*. Currently such a procedure is unavailable and hence the present investigations were undertaken on the following lines:

- Efficiency of different sanitation protocols on the microbial load and spoilage of Rohu (*Labeo rohita*) steaks after preparation and during refrigerated storage.
- The effect of marinating Rohu steaks in salt, acidulants and their combinations on the spoilage microflora and spoilage indices during refrigerated storage.
- The effect of spices on Rohu steaks pre marinated in salt and acidulant combinations on the spoilage indices during refrigerated storage.
- The performance of different flexible packaging materials in extension of shelf life of ready to cook fish steaks under commercial refrigerated conditions.

The results of the above are discussed and presented in this thesis.