Introduction
I. INTRODUCTION

Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted source of protein and other elements for the maintenance of healthy body (Andrew, 2001). The value of fish for human nutrition lies in the relatively high protein content, good digestibility, and high biological value (Geiger and Borgstorm, 1962). Apart from having superior quality of proteins, fish also contains essential omega-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3). Consumption of these fatty acids is believed to be beneficial to human health (Lee and Lip, 2003). The levels of the characteristic long chain polyunsaturated n-3 fatty acids (PUFA) make marine lipids unique compared to other lipid sources. These fatty acids are believed to play a preventive role in cardiovascular disease and in the alleviation of other health problems (Calder, 2004). The DHA is essential for human nutrition especially during pregnancy and lactation (Crawford, 2014). The land food chain is a poor source of DHA making marine lipids more special.

Indian mackerel (Rastrelliger kanagurta) a pelagic species belonging to the family Scombridae is found naturally and very abundantly in the west coast of India. The Indian mackerel fishery in west coast of India is commercially important fishery due to its food value and industrial uses. It contributes 7.2% to the total marine fish landing of India and forms the mainstay pelagic fishery after oil sardine. Indian mackerel as a fatty fish has all necessary proteins, vitamins and minerals in the desired proportions. In mackerel, the dark muscle is rich in lipids compared to light muscle. The lipid content of belly flap is remarkably higher compared to other parts. Commercial use of fatty fish species has been limited by the susceptibility of the fish to oxidative reaction of its lipids. In addition to the high concentration of highly unsaturated fatty acids, there also exist many pro-oxidants in
the muscle tissue of mackerel. The recorded landing of the fish for the year 2013 was 2.00 lakh tons (CMFRI, 2013). The consumption of Indian mackerel is either locally as fresh fish, iced or as frozen products. Generally frozen mackerel is exported to South-East Asian countries like Thailand, Malaysia and also to European countries, USA and UAE. India exported frozen Indian mackerel worth US $ 598,026 under HS Code 0302 with total quantity of 305,558 tons. Thailand is the largest buyer of frozen Indian mackerel accounting for exports worth US $ 364,078 followed by Malaysia and United States which imported frozen Indian mackerel worth US $ 193,631 and US $ 40,317 respectively (MPEDA, 2014). Average price of frozen Indian mackerel per unit is US $ 1.96. The market prospects in the international scenario too show that mackerel fishery is gaining importance.

Fish quality is very subjective nature and is very complex concept (Bremner 2000) which comprises biochemical, microbiological, nutritional and physiochemical attributes. The freshness of fish degrades after death due to various biochemical reactions such as changes in protein and lipid fractions and microbiological spoilage. This results in the deterioration of sensory quality and nutritional value of fish. Preservation of fish assumes greater importance to prevent the loss of nutritionally rich natural resource. Quality loss of fish may be caused by a wide range of reactions, some are predominantly chemical (e.g., oxidative rancidity, colour loss, and non-enzymic Maillard browning reactions), physical (e.g., movement of moisture, change in texture, evaporation of low molecular mass flavour compounds, and damage induced by freezing/thawing), enzymic (e.g., lipolytic rancidity, proteolysis, and enzymic browning reactions), and microbiological (i.e., food spoilage and food poisoning) (Huisveld, 1996). These various reactions are therefore the targets for fish preservation. Quality loss may be prevented or minimized by a range of techniques,
including the use of additives, in particular to interfere with oxidative chemical reactions and to prevent or delay microbial growth.

Nevertheless, fish, especially fatty fish is susceptible to oxidation, which is associated with the rancidity and loss in nutritive value (Hsieh and Kinsella, 1989; Frankel, 1998a). Apart from high amounts of PUFAs, the presence of haem pigments and trace amounts of metallic ions makes the fish, especially dark flesh fatty fish, more prone to lipid oxidation (Hsieh and Kinsella, 1989). Haemoglobin is also known as an important catalyst of lipid oxidation. Haemoglobin can be a source of activated oxygen, due to haemoglobin autoxidation, the haem or iron can be released from the protein to promote lipid oxidation (Richards and Hultin, 2002). Lipid oxidation can be accelerated by reduction in pH and could be due to enhanced autoxidation of haemoglobin at reduced pH (Tsuruga et al., 1998). Oxidation can also cause other detrimental effect such as discoloration, vitamin destruction and decomposition of essential fatty acids, leading to organoleptic failure and a decrease in nutritive value (Sherwin, 1978). To retard such a quality loss, synthetic antioxidants have been used to decrease lipid oxidation during the processing and storage of fish and fish products (Boyd et al., 1993). However, the use of synthetic antioxidants has raised questions regarding food safety and toxicity (Chang et al., 1977). The use of natural antioxidants is emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences. Food manufacturers are subjected to increased pressure on either to completely remove chemical preservatives from their food products or to adopt more “natural” and “healthy” alternatives for the maintenance or extension of a product’s shelf life (Nychas, 1995). There is considerable interest in the possible use of natural alternatives as phenolic compounds from plant sources either to prevent the growth of food borne pathogens or to delay the oxidative deterioration of foods. Presence of phenolics in
foods may have an important effect on the oxidative stability and microbial safety of products. In addition, many phenolics in foods possess important biological activity related to their inhibitory effects on metagenesis and carcinogenesis (Newmark, 1992).

Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety in fish too. Concern over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreaks of food borne disease (Tauxe, 1997). The use of natural antimicrobial compounds in food has gained much attention by the consumers and the food industry. This is primarily due to two major factors. First, the misuse and mishandling of antibiotics has resulted in the dramatic rise of a group of microorganisms including food borne pathogens that are not only antibiotic resistant but also more tolerant to several food processing and preservation methods. In addition, increasing consumers' awareness of the potential negative impact of synthetic preservatives on health versus the benefits of natural additives has generated interest among researchers in the development and use of natural products in foods. This has prompted the food industry to look for alternative preservatives that can enhance the safety and quality of foods. Compounds derived from natural sources have the potential to be used for food safety due to their antimicrobial properties against a broad range of food borne pathogens.

Grape seed is a by-product of the food industry, which is usually discarded as a waste and as such, presents a cheap source of natural antioxidant due to its phenolic content (Spigno and De Faveri, 2007). Seeds constitute a considerable proportion of the grape, ranging from 38–52% on a dry matter basis and constitute 46-69% polyphenols (Amerine and Joslyn, 1967). The antioxidant activity of grapes has been positively associated with their phenolic composition such as anthocyanins, flavonols, flavan-3-ols, procyanidins, and
phenolic acids (Bertelli et al., 2004; Fujii et al., 2007; Kedage et al., 2007; Pazos et al., 2005; Shafiee et al., 2003). These compounds have been shown to reduce hydroperoxide formation and inhibit lipid and protein oxidation (Heinonen et al., 1998). In addition to antioxidant properties, the grape seed phenolics have also displayed antimicrobial properties (Palma and Taylor, 1999). The antimicrobial property of grape seed extract is due to core structure with 3,4,5-trihydroxy phenyl groups found in epigallocatechin, epigallocatechin-3-ogallate, prodelphinidin and castalagin (Tagurt et al., 2004).

Papaya (Carica papaya L.) is native of America but has now spread all over the tropical world. The central cavity contains large quantities of seeds that comprise about 15% of the wet weight of the fruit (Pokorny, 2003). The total global production of papaya averages about 10.0 mmt, and India and Brazil are the major producers with annual production of 3.6 and 1.9 mmt, respectively. The fruit is rich in phytochemical, especially carotenoids and polyphenols (Sancho et al., 2007). Besides its juicy pulp, the peel and seeds of papaya are valuable too. Papaya seeds are known to give several health benefits (Adebiyi et al., 2003). Although papaya peel and seeds have various uses, the phytochemicals especially phenolic compounds in these parts of papaya have both antioxidative and antimicrobial properties. Despite of abundance of grape and papaya seeds in India, the extract from seed, has not been fully exploited, which can be used as an alternative natural antioxidant and antimicrobial agents in different food products.

Many researchers have attempted to extract the phenolic compounds from different plant origin and research on phenolic compounds from natural sources is catching up but there are very few work carried out on how to use the potential of natural antioxidants and antimicrobials from horticultural waste such as grape and papaya seeds in controlling/mitigating autoxidation and microbial spoilage of fish and fish products and
thereby extending their shelf life in our country. Hence the present investigation is a focused approach towards maximizing the use of phenolic compounds on oxidative and microbial spoilage of Indian mackerel during different storage conditions.

**The objectives of the investigation are:**

1. To extract natural antioxidants and antimicrobial substances from grape and papaya seeds.
2. To study antioxidant and antimicrobial properties of both the extracts.
3. To study the stability and shelf life characteristics of grape and papaya seed extract.
4. To study the efficacy of grape and papaya seed extract on quality of Indian mackerel steaks during different storage conditions.
5. To study the shelf life characteristics of Indian mackerel in whole form treated with grape and papaya seed extract under different storage conditions.