Review of literature
II. REVIEW OF LITERATURE

In the search for effective natural antioxidant and antimicrobial compounds based on horticultural waste to control oxidative and microbial spoilage of seafood products, it is crucial to understand the complex mechanisms of lipid oxidation in fish muscle and the chemical and physical factors influencing the effectiveness of antioxidants. The knowledge on the chemical structure of seed polyphenols and extraction methods is a prerequisite for the development of antioxidant and antimicrobial compounds from horticultural waste.

In the present review an attempt has been made to give comprehensive accounts on the lipid oxidation, role of antioxidants in prevention of lipid oxidation and its mechanism, factors affecting lipid oxidation, impact of lipid oxidation on rancidity, antioxidant and antimicrobial properties of grape and papaya seed extracts, phenolic compounds as antioxidants, extraction of plant phenolic compounds, stability of phenolic compounds and effect on the quality of fish with phenolic compounds during different storage conditions.

2.1. Lipid oxidation in sea food

Lipid oxidation is one of the major causes of quality deterioration in natural and processed sea foods. Oxidative deterioration is a large economic concern in the fish industry because it affects many quality characteristics such as flavor (rancidity), color, texture and the nutritive value of fish. In addition it produces potentially toxic compounds (Narwar, 1996). The lipid oxidation is one of the major processes that limit the shelf life of fish. In addition, the oxidative instability of polyunsaturated fatty acids often limits their use as nutritionally beneficial lipids in functional foods.

Lipid oxidation is a general term used to describe a complex sequence of chemical interactions between unsaturated fatty acyl groups in lipids with active oxygen (Narwar, 1996). The unsaturated fatty acid on triacylglycerols and phospholipids have low volatility
and do not directly contribute to aroma of fish. However, these fatty acids will decompose during lipid oxidation to form small, volatile molecules that produce the off-.aromas associated with oxidative rancidity. The mechanism of lipid oxidation in a particular food depends on the nature of the reactive species present in their physiochemical environment (Decker, 1998b). Thus a thorough understanding of lipid oxidation mechanisms is important in developing practical method for controlling lipid oxidation in sea foods.

2.1.1. Mechanism of lipid oxidation

The oxidation of lipids occur by a free radical chain reaction involving three processes: (1) initiation: the formation of free radicals; (2) propagation: the free radical chain reactions; and (3) termination: the formation of non-radical products (Frankel, 2005; Nawar, 1996) as shown in Fig.1. In the initiation step, a lipid free radical known as the alkyl radical (L•) is formed. Abstraction of a hydrogen atom from the central carbon of the pentadiene structure is found in most fatty acid acyl chains containing more than one double bond.

\[-\text{CH}=\text{CH}-\text{CH}-\text{CH}=\text{CH}^- + \text{H}^\bullet\]

The alkyl radical contains an unpaired electron that reacts rapidly with the oxygen biradical to form peroxyl radicals (LOO•) [2] in the propagation step. The following hydrogen transfer reaction that occurs with unsaturated lipids to convert the peroxyl radical to a hydroperoxide (LOOH) happens slower than the previous step [3]. Termination is the last step of autoxidation. In the termination stage [4-6], peroxyl radicals accumulate and react with each other forming non-radical products (Frankel, 2005; Erickson, 2002; Nawar, 1996; Ingold, 1962). In general, peroxide is not stable and can be decomposed to many products including acids, alcohols, aldehydes, ketones, etc. Those oxidation products could
undergo polymerisation, and might induce protein denaturation, leading to the changes in functional properties (Shahidi and Naczk, 2004).

Initiation: \( \text{In}^\bullet + \text{LH} \rightarrow \text{InH} + \text{L}^\bullet \) \[1\]

Propagation: \( \text{L}^\bullet + \text{O}_2 \rightarrow \text{LOO}^\bullet \) \[2\]

\( \text{LOO}^\bullet + \text{LH} \rightarrow \text{LOOH} + \text{L}^\bullet \) \[3\]

Termination: \( \text{LOO}^\bullet + \text{LOO}^\bullet \rightarrow \text{LOOL} + \text{O}_2 \) \[4\]

\( \text{L}^\bullet + \text{LOO}^\bullet \rightarrow \text{LOOL} \) \[5\]

\( \text{L}^\bullet + \text{L}^\bullet \rightarrow \text{LL} \) \[6\]

Where,

In\(^\bullet\)- initiator, LH- unsaturated fatty acid, L\(^\bullet\)- alkyl radical, LOO\(^\bullet\)- peroxyl radical

LOOH- hydroperoxide, LL- non radical product

2.2. Factors affecting lipid oxidation

The extent of oxidation can be influenced by both intrinsic and extrinsic factors such as the concentration of prooxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, oxygen consumption reaction and the fatty acid composition of the meat (Castell et al., 1965; Nawar, 1996; Undeland et al., 2003). Fish, in particular, contain higher levels of unsaturated lipids than those of mammals and birds. Thus, fish lipids undergo more rapid oxidation after capture, even at low temperature storage (Foegeding et al., 1996; Pacheco-Aguilar et al., 2000). Lakshmisha et al. (2014) reported that shelf life of Indian mackerel is limited due to oxidation of lipids, as shown by the increase in peroxide value (PV) during storage period of 12 h. Sohn et al. (2005) have reported that, the total lipid hydroperoxide content of Pacific saury (Cololabis saira), Japanese Spanish mackerel (Scomberomorus niphonius) and chub mackerel (Scomber japonicus) tend to increase in both dark and ordinary muscle throughout 4 d of iced storage. The concentration of ferrous
iron and its ability to activate the lipid oxidation reaction will be a key factor causing differences in lipid oxidation among species. In general, dark meats tend to have more reactive iron. Chaijan et al. (2004) have observed that, the lipid and myoglobin contents were higher in dark muscle than in ordinary muscle of both mackerel and sardine. Other constituents of meat including enzymatic and non-enzymatic reducing systems can accelerate oxidation by converting iron from the inactive ferric form to the active ferrous state (Foegeding et al., 1996). Apart from a plenty of unsaturated fatty acids, haem protein as well as reactive iron in the muscle contributed to the accelerated oxidation (Chaijan et al., 2006). Like most chemical reactions, lipid oxidation rates increase with increasing temperature and time. Saeed and Howell (2002) have reported that, the rate of lipid oxidation in frozen mackerel increased with increasing storage time and storage temperature. NaCl is also able to catalyze lipid oxidation in muscle tissue (Foegeding et al., 1996). Alternatively, the Na⁺ may replace iron from a cellular complex via an ion exchange reaction (Kanner and Kinsella, 1983). The displaced iron may then participate in the initiation of lipid oxidation. It is most likely that meat or meat products containing salt such as surimi and cured meat are susceptible to lipid oxidation (Chaijan, 2008).

2.3. Impact of lipid oxidation on rancidity

Rancidity in food occurs when unsaturated fatty acids decompose into volatile compounds. These volatile oxidation products are produced from the decomposition of fatty acid hydroperoxides (Frankel, 1998a). Fatty acid hydroperoxides produced during propagation do not have a direct adverse effect on the flavor and aroma of foods. However, lipid hydroperoxide decomposition produces alkoxyl radicals (LO•), which in turn can cause the decomposition of the fatty acid. β-scission reactions occur after hydroperoxides decompose into alkoxyl radicals. These highly energetic alkoxyl radicals have the ability to
abstract an electron from the carbon-carbon bond on either side of the oxygen radical in order to cleave the fatty acid chain. Hydroperoxide is a primary oxidation product during fish storage which is readily decomposed to a variety of volatile compounds including aldehydes, ketones and alcohols (Frankel et al., 1994). The β-scission reaction is important because it causes fatty acids to decompose into low molecular weight, volatile compounds (Decker et al., 2005; Chaiyasit et al., 2007). The aldehydes and ketones produced from the β-scission reaction are the source of the characteristic rancid flavors and aromas in foods (Coleman and Williams, 2007). Human olfactory receptors usually have remarkably low organoleptic thresholds to most of these volatile compounds (Ke et al., 1975). Rancid or fishy odor has been identified as a common off-flavor associated with fish flesh and directly related with the formation of the secondary lipid oxidation products (Ke et al., 1975; Sohn et al., 2005). Varlet et al. (2006) reported that carbonyl compounds, such as heptanal or (E, Z)-2, 6-nonadienal, show a high odorant intensity in salmon (Salmo salar), giving the flesh its typical fishy odor. The fishy volatiles identified in the boiled sardine were dimethyl sulfide, acetaldehyde, propionaldehyde, butyraldehyde, 2-ethylfuran, valeraldehyde, 2, 3-pentanedione, hexanal and 1-penten-3-ol (Kasahara and Osawa, 1998). Rancid odor is often related to a significant number of volatile compounds that can be produced from oxidation of polyunsaturated fatty acids (PUFA). Aldehydes are the main volatile secondary oxidation products responsible for off-flavors and odors during storage and treatments of foods. Several volatiles have been associated with the characteristic odors and flavors of oxidized fish, described as rancid, painty, fishy and cod-liver like (Pearson et al., 1977). Oxidation of unsaturated fatty acids in fish was related to the formation of E-2-pentenal, E-2-hexenal, Z-4-heptenal, (E, E)-2, 4-heptadienal and 2, 4, 7-decatrienial (Frankel, 1998a). Other volatiles formed during oxidation of fish lipids are 1-
penten-3-ol, 1-octen-3-ol, 1, 5-octadien-3-one and 2, 6-nonadienal, some of them having high odor impact (Milo and Grosch, 1993). Fish volatiles have been conventionally analyzed by gas chromatographic (GC) techniques. Simultaneous steam distillation with solvent extraction has been employed for determining volatiles in fish muscle, but it is time- and solvent-consuming, which may result in the loss or degradation of some of the volatile compounds (Prost et al., 1998). Analysis of volatiles in fish and seafood has been widely performed by several headspace techniques (Girard and Nakai, 1994; Medina et al., 1999; Frankel, 2007; Alasalvar et al., 2005). Both, dynamic headspace and purge-and-trap (DHS techniques) coupled with gas chromatography have been extensively used for the analysis of aroma compounds in fish muscle and provided better sensitivity and efficacy than static head space (Iglesias and Medina, 2008).

Incorporation of antioxidants into foods is one of the most effective methods of retarding lipid oxidation. However, many factors can impact the activity of antioxidants with some antioxidants retarding lipid oxidation under certain conditions but promoting lipid oxidation under other conditions (Huang et al., 1997).

2.4. Antioxidants

Antioxidants are substances, synthetic or naturally occurring, that can delay the onset or slow the rate of oxidation of autoxidizable materials. Antioxidants are regarded as compounds capable of delaying, retarding or preventing autoxidation processes. According to the USFDA Code of Federal Regulations, “antioxidants are substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation” (Dziezak, 1986). It has been suggested that an ideal food-grade antioxidant should be safe, not impart color, odor or flavor, be effective at low concentrations, be easy to incorporate, survive after processing, and be stable in the finished product as well as available at a low cost.
(Coppen, 1994). The activity of antioxidants is strongly influenced by numerous factors. Thus, compounds that are effective antioxidants in one system may be unsuitable in other systems. Some factors influencing antioxidant activity are the nature of the lipid substrate, the hydrophilic-lipophilic balance of the antioxidant, physical and chemical environments and interfacial interactions (Chang et al., 2003; Portet, 1993). Antioxidants act at different levels in the oxidative sequence involving lipid molecules. They may decrease oxygen concentration, intercept singlet oxygen, prevent first-chain initiation by scavenging initial radicals such as hydroxyl radicals, bind metal ion catalysts, decompose primary products of oxidation to non-radical species and break chains to prevent continued hydrogen abstraction from substrates (Shahidi, 2002). According to their mechanism of action, antioxidants can be classified as primary or secondary antioxidants. Primary antioxidants are chain breaking antioxidants and can inhibit lipid oxidation by interfering at the propagation or initiation phase or in β-scission reactions by accepting free radicals to form stable free radicals. Secondary antioxidants are considered preventative antioxidants, such as chelators, oxygen scavengers and singlet oxygen quenchers. These antioxidants decrease the rate of oxidation through numerous mechanisms; however, they do not convert free radicals into more stable products (Chaiyasit et al., 2007).

2.4.1. Synthetic antioxidants

Synthetic antioxidants are man made and are used to stabilize fats, oils, and lipid containing foods and are mostly phenolic-based. Many compounds are active as antioxidants, but only a few are incorporated into food because of strict safety regulations. These phenolic derivatives usually contain more than one hydroxyl or methoxy group. Presently almost all processed foods have synthetic antioxidants incorporated, which are reported to be safe, although some studies indicate otherwise. Synthetic phenolic
antioxidants are p-substituted, whereas the natural phenolic compounds are mostly o-
substituted. The p-substituted substances are preferred because of their lower toxicity. The
m-substituted compounds are inactive. Synthetic phenolic antioxidants are always
substituted with alkyl groups to improve their solubility in fats and oils and to reduce their
toxicity (Gordon, 1990). The primary mechanism of activity of these antioxidants is similar
to those of primary antioxidants. An antioxidant molecule reacts with a peroxy radical
produced by the oxidizing lipid, thus forming a hydroperoxide molecule and an antioxidant
free radical. The following are some of the synthetic antioxidants generally used in food
system.

2.4.1.1 Butylated Hydroxy Anisole

Butylated hydroxy anisole is a monophenolic compound that exists as a mixture of
two isomers viz, 3-tertiary-butyl-4-hydroxyanisole (90%) and 2-tertiary-butyl-4-
hydroxyanisole (10%) as shown in Fig. 2. The 3-isomer shows a higher antioxidant activity
than the 2-isomer. BHA is commercially available as white, waxy flakes that are lipid
soluble.

2.4.1.2 Butylated Hydroxy Toluene

It is chemically 2, 6-di-tert-butyl-p-cresol (DBPC) which is a white crystalline solid
with a faint characteristic odor as depicted in Fig. 2. It is insoluble in water and propylene
glycol, but it is freely soluble in alcohol. BHT is obtained by alkylation of p-cresol with
isobutene or by monobutylation of m-p-cresol mixtures. BHT is used as a chemical
antioxidant for food, cosmetics and pharmaceuticals much like BHA.

2.4.1.3 Propyl Gallate

Propyl gallate is the n-propyl ester of gallic acid (3, 4, 5-trihydroxybenzoic acid) as
shown in Fig 2. It is soluble in ethanol, ethyl ether, oil, lard and aqueous solutions of
polyethylene glycol (PEG) ethers of acetyl alcohol. It is only slightly soluble in water. Currently propyl gallate is being used as an antioxidant in a number of cosmetic products at maximum concentrations of 0.1%. It is generally recognized as safe antioxidant (GRAS) to protect fats, oils and fat containing foods from rancidity that results from the formation of peroxides (Becker, 2007).

2.4.1.4. Ethoxyquin

Ethoxyquin, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, is used as an antioxidant in animal feeds primarily to protect carotenoid oxidation. The structure of ethoxyquin is shown in Fig 2. It may also be used in fish products, fish oil, poultry fats, potatoes, apples, and pears during storage. Ethoxyquin may act as a free radical terminator.

2.4.1.5. Tertiary-Butyl Hydro Quinone (TBHQ)

This is a diphenolic antioxidant and is widely used in a variety of fats and oils as shown in Fig 2. TBHQ has excellent carry-through properties and is a very effective antioxidant for use in frying oils. It is available as a beige color powder that is used alone or in combination with BHA or BHT. TBHQ can be used in a variety of lipid-containing foods and fats and oils. Chelating agents such as monoacylglycerols and citrates enhance the activity of TBHQ, mainly in vegetable oils and shortenings.

2.4.2. Natural Antioxidants

Considering the possibility of undesirable influences of oxidized lipids on the human beings, it is of great importance to minimize the content of products of lipid oxidation in food. In industrial processing, mainly synthetic antioxidants are used, in order to prolong the storage stability of food. Synthetic antioxidants like BHA and BHT have been shown to exert carcinogenic effects in humans (Ames, 1983; Baardseth, 1989). There is a general trend toward replacing the use of synthetic antioxidants in food processing by
the use of natural oxidation inhibitors or by the preferential use of ingredients that naturally possess antioxidant activity. Natural antioxidants are primarily plant phenolics that may occur in all parts of plants, such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt and Hudson, 1990). Plant phenolics are multifunctional and can act as reducing agents, free radical terminators, metal chelators and singlet oxygen quenchers (Mathew and Abraham, 2006). The chemical structures of plant phenolic compounds are related to those of synthetic antioxidants. Natural extracts such as grape seeds, sage, citrus peel, sesame seed oil and rosehip have the same and sometimes even better antioxidative characteristics (Tang et al., 2001).

2.5. Sources of natural phenolic compounds

Different phenolic compounds with antioxidative activity from various natural sources have been reported. Flavonoids are the most widely studied class of polyphenols with respect to their antioxidant and biological activities. They have powerful antioxidant activities in vitro, being able to scavenge a wide range of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and chlorine species, such as superoxide, hydroxyl and peroxyl radicals, and peroxynitrous acid and hypochlorous acid (Hernandez et al., 2009). Different antioxidative compounds have been identified in the various natural products as shown in Table 1.

2.5.1. Grape seed as natural phenolic compound

Grape seed is the most abundant source of grape polyphenols, mostly in the form of gallic acid and its catechin derivatives. The major grape seed catechins are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC). In general, approximately 30% of the dry weight of grape seed is catechin (Ho et al., 2009). These polyphenol compounds have been proved to show various
biological effects such as antioxidant or antimicrobial (Baydar et al., 2004; Jayaprakasha et al., 2003). In fact, seed extract has recently been marketed as a dietary supplement and advocated for its beneficial antioxidant effects and free radical-scavenging ability (Singh and Agarwal, 2006). The multiple mechanisms of their antioxidative activity are expressed in its ability of radical scavenging, metal chelation, and synergism with other antioxidants (Lu and Foo, 1999).

The antioxidant properties of GSE are primarily due to flavonoids that can perform scavenging action on free radicals (superoxide, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl (DPPH)), metal chelating properties, reduction of hydroperoxide formation and their effects on cell signaling pathways and gene expression (Jacob et al., 2008; Saito et al., 1998). The presence of the functional group “–OH” in the structure and its position on the ring of the flavanoid molecule determine the antioxidant capacity as shown in Fig. 3 (Arora et al., 1998). Addition of “–OH” groups to the flavonoid nucleus will enhance the antioxidant activity, while substitution by –OCH$_3$ groups diminishes the antioxidant activity as shown in Fig.3 (Majo et al., 2008). Degree of polymerization of the procyanidins may also determine the antioxidant activity as the higher the degree of polymerization, the higher the antioxidant activity (Spranger et al., 2008). Among the different parts of grape plant, seeds exhibit highest antioxidant activity followed by the skin and the flesh (Pastrana-Bonilla et al., 2003). The antioxidant potential of GSE is twenty and fifty fold greater than those of vitamins E and C respectively (Shi et al., 2003). Flavonoid components obtained from grape by-products were effective in retarding lipid oxidation in different systems containing fish lipids, including bulk oil, oil-in-water emulsion and frozen mackerel muscle (Pazos et al., 2005). Grape phenolics showed high solubility in the aqueous media and low in oily media. Grape antioxidant dietary fibre
(GADF) containing high amounts of dietary fibre and phenolics antioxidants such as phenolics acids, anthocyanidins, proanthocyanidins, catechins and other flavonoids, was successfully used as an ingredient in minced fish (Sanchez-Alonso et al., 2007).

2.5.2. Papaya seed as natural phenolic compound

Papaya (Carica papaya L.) is native of tropical 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 (Desai and Wagh, 1995). Although papaya peel and seeds have various uses, the phytochemicals especially phenolic compounds in these parts of papaya have antioxidative properties (Jorge and Malacrida, 2009). The fruit and seeds of Carica papaya are rich in polyphenols, carotenoids, phenolic acids, flavanols, isothiocyanates, pectin, papain, citric acid and ascorbic acid (Sancho et al., 2007). Antioxidant activities of the ethanol, petroleum ether, ethyl acetate, n-butanol and water extract fractions from the seeds of papaya was investigated by Zhou et al., (2011) and result of the study revealed that ethyl acetate fraction showed the strongest DPPH and hydroxyl free radicals scavenging activities, and its activities were stronger than those of ascorbic acid and sodium benzoate. The chemical structures present in papaya seed as reported by Zhou et al. (2011) for the first time were p-hydroxybenzoic acid and vanillic acid which are widely found in fruits and vegetables have strong antioxidant activities. Maisarah et al. (2013) had studied the relation between total phenolic content and antioxidant capacity from different parts of papaya and the results of the study showed that there is significant relation between total phenolic content and antioxidant capacity, and total antioxidant activity were higher in leaves compared to seeds and ripe fruit. Radical scavenging activity of the papaya seed extract depends on the polarity of the solvent as reported by Kothari and Seshadri (2010) and the results of the experiment revealed that
antioxidant activity of 3179.66 g gallic acid equi/g of dry extract of papaya seed. Antioxidant capacity is the ability of antioxidant to minimize the formation of hydroperoxides in lipid model system. The antioxidant capacity of various parts of papaya fruit were measured in terms of β-carotene lipid model system by Maisarah et al., (2013) and results of the investigation revealed that highest antioxidant activity among the samples was observed in unripe papaya whereas seeds had the lowest antioxidant activity of 58%.

2.6. Role of antioxidants in the prevention of lipid oxidation

Unsaturated lipids in meats are very susceptible to chemical attack by oxygen. The addition of antioxidants is a method of increasing shelf life, especially of lipids and lipid containing products. Antioxidants can interact with free radicals that start chain reactions to damage compounds. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by getting oxidized themselves (Yerlikaya and Gokoglu, 2010). Antioxidants may exert their inhibitory effect against oxidation via different mechanisms and with varied activities. They may be broadly classified based on their mode of action into primary antioxidants which break the chain reaction of oxidation by scavenging free radical intermediates, and secondary antioxidants, which prevent or retard oxidation by suppression of oxidation initiator or accelerators or regeneration of primary antioxidants. Primary antioxidants such as most phenolic compounds are able to neutralize free radicals by donating a hydrogen atom as shown in Fig.4. Primary antioxidants can trap two lipid radicals by donating a hydrogen atom to one radical and receiving an electron from another radical to form stable non-radical products (Shahidi and Zhong, 2010).

Secondary antioxidants prevent or retard oxidation by several mechanisms. They exert their inhibitory effect by suppressing the oxidation promoters, including metal ions
singlet oxygen, prooxidative enzymes and other oxidants. Metal ions are known to act as catalysts of oxidation reaction producing free radicals through electron transfer. Metal chelators such as citric acid, phosphoric acid and ethylene diamine tetra acetic acid (EDTA) can decrease the pro-oxidant effect by forming a thermodynamically stable complex and reducing their redox potentials (Shahidi and Zhong, 2010).

2.6.1. Free radical scavengers and chain breaking antioxidants

Free radical scavengers and chain breaking antioxidants have the ability to slow or inhibit oxidation by interfering with either chain initiation and/or propagation. The following reaction demonstrates the ability of free radical scavengers (FRS) to interact with either peroxyl (LOO•) or alkoxyl (LO•) radicals (Decker et al., 2005; Frankel, 2005).

\[ \text{LOO}^\cdot \text{ or LO}^\cdot + \text{FRS} \rightarrow \text{LOOH} \text{ or LOH} + \text{FRS}^\cdot. \]

Peroxyl radicals are found in the greatest concentration of all radicals in a system and have lower energy than other radicals. Therefore, they preferentially react with the low energy hydrogens of the free radical scavenger rather than the unsaturated fatty acid, resulting in the formation of a free radical scavenger radical (FRS•). The resulting low energy FRS• will be less likely to catalyze the oxidation of unsaturated fatty acids. The inactivation of the FRS• occurs during a termination reaction with another FRS• or lipid radical (Decker et al., 2005; Buettner; 1993; Frankel, 2005). Free radical scavengers can be physically classified into two groups: 1. hydrophilic (water loving/polar) and 2. lipophilic (oil loving/non-polar). The difference in the behavior of these two types of FRS in food systems is referred to as the antioxidant polar paradox. The premise of this theory is based on the observation that, in emulsified oils, non-polar FRS are more effective than polar FRS, while polar FRS are more effective than non-polar FRS in bulk oils (Frankel, 2005; Chaiyasit et al., 2007; Portet, 1993; Decker, 1998b). The key to this phenomenon is the
ability of the FRS to concentrate where lipid oxidation is most prevalent. Polar FRS concentrate at oil-air or oil-water interfaces in bulk oils, where the majority of oxidation occurs due to high concentrations of oxygen and prooxidants. In emulsions, non-polar FRS accumulates in the lipid phase and at the oil-water interface where interactions between hydroperoxides at the droplet surface and prooxidants in the aqueous phase occur (Decker, 1998b; Chaiyasit et al., 2007; Decker et al., 2005). To be used in food applications, synthetic FRS must be sufficiently active enough to be used at low concentrations (below 0.02%) and are not toxic. They must also be stable to processing and cooking conditions. Compared to natural FRS, synthetic FRS are more effective, can be used at lower concentrations, are less expensive and can be prepared with consistent quality without an effect on flavor, color and aroma of the food product (Frankel, 2005; Pokorny, 2003). However, synthetic FRS are “label unfriendly” additives (Chaiyasit et al., 2007). Some of the most commonly used synthetic FRS in food systems are propyl gallate, butylated hydroxyl toluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) (Decker et al., 2005; Frankel, 2007). In the past couple of decades; use of natural FRS has increased due to worries about the possible hazardous effects of synthetic FRS and also current trends against the use of regulated/artificial food additives. The benefits of using natural FRS include GRAS status, allowance to use higher concentrations and worldwide acceptance. The negative side of natural FRS includes wide variation in concentration of active components due to source and extraction methods and undesirable effects on flavor, color and aroma of foods (Frankel, 2005; Pokorny, 2003).

Potentially active compounds from natural sources such as fruits, herbs, roots, bark and leaves have been extensively studied, since there is much interest on their FRS activity in relation to human health. Natural compounds that possess FRS activity are polyphenols,
such as flavonoids, bioflavonoids, isoflavones, and tannins, as well as some vitamins including vitamin A, C and E. The role of these compounds is to interrupt the free radical chain reaction involved in oxidation. Polyphenols have strong FRS properties which can help protect cells against adverse effects of reactive oxygen species, free radicals and pro-oxidative metal ions (Dufresne and Farnworth, 2001; Aviram et al., 2002). Of the tea catechins, green tea extracts and have been found to have higher phenol content and greater chain-breaking activity than black tea extracts (Manzocco et al., 1998). Carotenoids, found in fruits and vegetables, are another major group of natural compounds which have FRS properties. Lycopene has a high FRS potential due to its capacity to inactivate free radicals in lipid phases (Ribeiro et al., 2003) and by interfering with reactions of damaging oxidizing agents and free radicals (Ribeiro et al., 2003; Henry et al., 1998).

2.6.2. Metal inactivators and chelators

Metal inactivators and chelators are compounds that can inhibit lipid oxidation by mechanisms that do not involve the deactivation of free radical chains. These metal inactivators decrease the ability of metal ions to promote initiation reactions and the decomposition of hydroperoxides into secondary aldehydes (Frankel, 2005; Pokorny, 2003). Chelators inhibit metal-catalyzed reactions by: prevention of metal redox cycling, formation of insoluble metal complexes, steric hindrance of metal-lipid interactions or oxidation intermediates (e.g. hydroperoxides) and/or occupation of all metal coordination sites (Decker, 1998a). Most chelators accumulate in the aqueous phase of foods, however, in order to inactivate lipid-soluble metals, some chelators must also partition into the lipid phase (Decker et al., 2005). Conversely, under certain conditions, some chelators can increase metal solubility or alter the redox potential of metals, thus increasing oxidative
reactions (Decker, 1998a). Ethylene diamine tetra acetic acid (EDTA), one of the most effective metal chelators, along with citric, tartaric and phosphoric acids is compounds which can deactivate metals by forming stable coordination complexes with pro-oxidant metals, thus effectively inhibiting both metal-catalyzed initiation and decomposition of hydroperoxides (Frankel, 2005). However, the antioxidative and/or pro-oxidative properties of metal chelators are often concentration dependant. It has been found that, when present at an EDTA: iron ratio of > 1, EDTA will perform as a strong metal chelator, in contrast, at an EDTA: iron ratio of ≤ 1, EDTA can behave as a prooxidant (Mahoney and Graf, 1986).

2.6.3. Singlet oxygen quenchers

The use of quenching agents is an effective way to reduce singlet oxygen oxidation. Quenching agents may decrease singlet oxygen promoted oxidation by quenching the excited triplet sensitizer or singlet oxygen by chemical or physical means. Chemical quenching involves the reaction of singlet oxygen with the quenching agent to produce stable products. Physical quenching returns singlet oxygen to triplet oxygen without the consumption of oxygen and any chemical changes of quenching agent (Min and Boff, 2002; Frankel, 2005). Natural food components such as carotenoids, tocopherols and ascorbic acid have been found to be effective quenching agents (Min and Boff, 2002). Carotenoids can chemically quench singlet oxygen when the singlet oxygen attacks the double bonds of the carotenoid, resulting in carotenoid breakdown products such as aldehydes and ketones. Physical quenching does not lead to breakdown products. During physical quenching, there is a transfer of energy from the singlet oxygen to the carotenoid, producing an excited state carotenoid and ground state triplet oxygen. The energy from the excited carotenoid is dissipated by vibrational and rotational interactions with the
surrounding solvent to return it to the ground state (Decker et al., 2005). Lycopene has been found to be one of the most efficient singlet oxygen quenchers of the biological carotenoids (Dimascio et al., 1989).

2.6.4. Multiple antioxidant functions

Antioxidant compounds may reinforce each other in multi-component systems by cooperative effects known as “synergism”. Synergism imparts more protection against lipid oxidation than the sum of the activities of the compounds when used separately (Coleman and Williams, 2007). In addition, the use of synergistic antioxidant mixtures can allow for a reduction in the concentration of each antioxidant (Abdalla and Roozen, 1999). If both initiation and propagation are suppressed, successful synergistic inhibition can be achieved. A commonly used combination of synergistic compounds in foods is pairing of metal inactivators with chain breaking antioxidants (Nawar, 1996; Frankel, 2005). An example of synergism between two compounds is the combined antioxidative effect of ascorbic acid and butylated hydroxy toluene (BHT). Ascorbic acid has the capability to chelate metals, therefore limiting their ability to initiate lipid oxidation. BHT, a phenol and a chain breaking antioxidant, has been shown to be much more effective at retarding lipid oxidation in the presence of ascorbic acid (Coleman and Williams, 2007). Recently, the synergistic antioxidant effect between rosemary extract and BHT was studied and it was found that a comparable antioxidant activity of rosemary, BHT and α-tocopherol was considerably higher than BHT (Romano et al., 2006). This indicated the positive interaction between rosemary extract and BHT on increasing the total antioxidative activity. Rosemary methanolic extract enhances the antiradical efficiency of BHT through synergistic interactions (Romano et al., 2006). Positive interactions of the rosemary methanolic extract with ascorbic acid and α-tocopherol on antioxidative efficiency was
also reported by (Romano et al., 2006). Several studies have shown that plant polyphenols have a synergistic effect with other antioxidants present in plant material (Graversen et al., 2008). Lettuce extract had a clear antioxidative effect as evidenced by a lag phase for formation of conjugated dienes and α-tocopherol and especially quercetin acted synergistically in prolongation of the lag phase both following initiation in the lipid phase and in the aqueous phase (Altunkaya et al., 2009). Antioxidants localised at or near the interface of the liposomes such as quercetin and α-tocopherol acted synergistically with lettuce extract as an antioxidant, while the hydrophilic antioxidant ascorbic acid showed no synergism (Altunkaya et al., 2009). Synergistic interactions with respect to antioxidant activity and biological functions can also be found between flavonoids such as soy and green tea (Bertipaglia et al., 2008).

2.7. Microbial spoilage associated with seafood

Microbial activity is another mode of deterioration of sea foods apart from lipid oxidation and is often responsible for the loss of quality and safety. Concern over pathogenic and spoilage microorganisms in sea food is increasing due to the increase in outbreaks of sea food borne disease (Tauxe, 1997). As fish products are perishable by nature they require protection from spoilage during their preparation, storage, and distribution to give them desired shelf life. Fish products can be subjected to contamination by bacteria and fungi. Many of these microorganisms can cause undesirable reactions that deteriorate flavor, odor, color, sensory, and textural properties of foods. Microbial growth is a major concern because some microorganisms can potentially cause food-borne illness.

To prevent growth of spoilage and pathogenic microorganisms in foods, several preservation techniques, such as heat treatment, salting, acidification, and drying have been used in the food industry (Farkas, 2007). Numerous efforts are conducted to find natural
alternatives to prevent bacterial and fungal growth in sea food industry. In recent years, because of the great consumer awareness and concern regarding synthetic chemical additives, fish preserved with natural additives have become very popular. To inhibit growth of undesirable microorganisms in fish products, the antimicrobials can be directly added into the product formulation, coated on its surface or incorporated into the packaging material. Direct incorporation of active agents into fishery products results in an immediate but short-term reduction of bacterial populations, while the antimicrobial films can maintain their activity for a long period of time (Hanusova et al., 2009).

Currently there is a growing interest to use natural antibacterial compounds, like plant extracts from seeds and spices for the preservation of foods, as these possess a characteristic flavour and sometimes show antioxidant activity as well as antimicrobial activity (Smid and Gorris, 1999). Many natural compounds from plants sources e.g., basil, thyme, oregano, cinnamon, clove, grape seed, and papaya seed and rosemary shows antimicrobial activity. In this context, phenolic compounds from plant sources are gaining a wide interest in food processing industry for their potential as decontaminating agents, as they are Generally Recognized as Safe. The antimicrobial activity of plant phenolic sources is due to their chemical structure, in particular to the presence of hydrophilic functional groups, such as hydroxyl groups of phenolic components and/or lipophilicity of some essential oil components (Dorman and Deans, 2000). Usually, the compounds with phenolic groups are grape seeds; papaya seeds, clove, oregano, rosemary, thyme, sage, and vanillin are the most effective. They are more inhibitory against Gram-positive than Gram-negative bacteria (Marino et al., 2001).

2.7.1. Mode of antimicrobial activity
Antimicrobial agents use different antimicrobial activities in which they may interfere with cell wall synthesis, inhibit protein synthesis, inhibit nucleic acid synthesis or block metabolic pathways to inhibit growth of microorganisms or eliminate them as shown in Fig. 5.

**Interference with cell wall synthesis:**

Antimicrobial agents can prevent cell wall synthesis, simply by blocking the synthesis of peptidoglycan layer which covers the outer surface of the cytoplasmic membrane.

**Interference with protein synthesis:**

A number of antibacterial agents act by inhibiting ribosome function. Bacterial ribosomes contain two subunits, the 50S and 30S subunits, binding to these sites cause protein chain termination and inhibit protein synthesis.

**Interference with cytoplasmic membrane:**

This type of antimicrobial agents play role in disruption and destabilization of the cytoplasmic membrane.

**2.7.2. Antimicrobial properties of grape seed extracts**

Grape seed extract is a rich source of polymers of flavan-3-ols like (+)-catechin and (−)-epicatechin, its antimicrobial properties can be attributed to the general mechanisms of phenolics. The core structures with 3, 4, 5-trihydroxyphenyl groups found in epigallocatechin, epigallocatechin-3-Ogallate, castalagin and prodelphinidin might be important for antibacterial activity (Tagurt *et al.*, 2004). Polyphenols extracted from grape seed extract have shown inhibitory effects on Gram-positive as well as Gram-negative bacteria (Gadang *et al.*, 2008). The susceptibility of GSE against various bacterial strains can be related to differences in cell membranes (Ikigai *et al.*, 1993). The antibacterial
activities of grape seed extract on various strains of Staphylococcus and Gram-negative rods including *E. coli, Klebsiella pneumoniae* and Salmonella was investigated by Yoda *et al.* (2004) and found that 50–100 μg/mL was required to inhibit growth of Staphylococcus and concentrations higher than 800 μg/mL was required to inhibit Gm - ve rods.

The catechins present in grape seed extract, is the most potent in exhibiting antimicrobial activity due to the galloyl moiety present in their structures (Shimamura *et al.*, 2007). The outer cell membrane or cytoplasmic membrane of a bacterium is essentially composed of a phospholipid bilayer and proteins and is the major site of interaction with antimicrobial compounds. Damage to this vital membrane can result in death of the bacterium and can occur in the following ways: (i) physical disruption of the membrane (Shimamura *et al.*, 2007); (ii) dissipation of the proton motive force (PMF) (Juven *et al.*, 1994a) and (iii) inhibition of membrane-associated enzyme activity. Functional hydroxyl groups and conjugated double bonds in the reactive groups of natural plant extracts may be involved in their binding to the cell wall components (usually proteins) (Mason and Wasserman, 1987). Catechins (galloyl and gallic moieties) have deteriorating effect on the lipid bilayer membrane that results in the loss of cell structure and function eventually leading to cell death (Cox *et al.*, 2001; Ikigai *et al.*, 1993; Tsuchiya *et al.*, 1996). Presence of gallic acid esters in grape seed extract are responsible for their high affinity for lipid bilayers, and affect the membrane structure (Hashimoto *et al.*, 1999). Major phenolic constituents like epicatechin, caffeic acid, benzoic acid and syringic acid may alter the cell morphology by influencing the osmotic pressure of the cell, thus disrupting the cytoplasmic membrane and causing leakage of cell constituents (Davidson and Naidu, 2000; Sivarooiban *et al.*, 2008a). Grape seeds extract was also reported for its potential of being a food preservative due to its antimicrobial activity (Rhodes *et al.*, 2006).
The increasing order of the antimicrobial activity reported in grape plant was flesh, fermented pomace, skin, leaves and seeds (Xia et al., 2010). Anastasiadi et al., (2009) have suggested that high concentration of flavonoids and their derivatives in grape seeds, flavonoids, stilbenes, and phenolic acids in grape stems were responsible for the antimicrobial activity. Vaquero et al., (2007) concluded that the non-flavonoid caffeic acid and the flavonoids such as rutin and quercetin had higher inhibitory activities on growth of Listeria monocytogenes. The effect of grape extracts for antibacterial activity by pour plate method against Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa was studied by Jayaprakasha et al., (2003). It was found that, Gm +ve bacteria were completely inhibited at 850–1000 ppm, while Gm-ve bacteria were inhibited at 1250–1500 ppm concentration.

2.7.3. Antimicrobial properties of papaya seed extract

The seeds of Carica papaya L. (Caricaceae) have been used for decades in parts of Asia and South America as vermifugical agent. In India, the seeds are administered with honey for expelling roundworms (Krishnakumari and Majumder, 1960; Adebiyi et al., 2003). In Panama, powdered papaya seeds mixed with honey and castor oil are orally taken by (as laxative) to get rid of intestinal worms (Gupta et al., 1979; Adebiyi et al., 2003). There are some studies showing that papaya seeds extracts are capable of killing worms such as Toxascaris transfuga, Ascaris lumbricoides, Pheretima sp. and Caenorhabditis elegans in vitro and also deworm infected animals (Adebiyi et al., 2003). Apart from their use as vermifugical agents, papaya seed preparations are reported to use in folk medicine to facilitate good menstrual flow and are thought to have abortifacient properties. Benzyl isothiocyanate (BITC), a chemopreventive phytochemical found in cruciferous vegetables has also been shown to be present in different extracts of papaya seeds (Adebiyi et al.,
Fruit and seed extracts have profound bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella flexneri* (Oloyede, 2005).

### 2.8. Limitations and safety issues

It must be stressed however, that natural antioxidants and antimicrobial also have various drawbacks that limit their large scale application in food industry. The antioxidant performance of natural compounds is generally less effective than the synthetic antioxidants and larger amounts of natural antioxidants need to be added to food to achieve the same effect. The actual antioxidant effectiveness depends to a large extent upon food composition and particular processing and storage conditions. Plant derived polyphenols are known to be prone to complex with proteins. Many of the phenolic rich extracts have intense characteristic odour, bitter taste and distinct colour, which adversely affect the sensory properties of food products (Gomez and Montero, 2007). Natural antioxidants have been generally considered safe for human consumption and the potential risk to human health has not been adequately investigated (Frankel, 2007). There is increasing evidence to suggest that caution should be exercised when assessing the safety of natural antioxidants.

### 2.9. Extraction of plant polyphenols

Extraction, pharmaceutically speaking, can be defined as separation of bioactive material from plants or animal tissues by utilizing selective solvent while conducting standard extraction procedures. These bioactive constituents can then be used in pharmaceutical, food or cosmetic industry. Both extraction yield and bioactivity of extracts strongly depend on the solvent, due to different, for example, antioxidant potential of compounds with different polarity (Moure *et al.*, 2001). During extraction processes, the
solvent is added to the sample and then removed by a proper method. This proper method either can be vacuum-drying, ultra filtration or evaporation. Method of choice can be dependent on solvent cost. If the cost is high, recovery systems such as rotary evaporators can be used to remove the solvent from extract solution. The material in which the extracts will be used is an important issue that needs to be taken into account. If the solvents will be used in extraction of food additives and biological materials they should be;

• Nontoxic
• Having high capacity
• Having high distribution coefficient
• Highly selective for the solutes
• Easily recoverable
• Stable and inert
• Environmentally safe
• Inexpensive
• Nonflammable
• Nonexplosive

Equilibrium state and mass transfer rate are two aspects that control the extraction process (Shi et al., 2005). In solvent extraction process of phenolic material from their solid hosts, there are two essential stages; dissolution of phenolic compounds in plant matrix and their diffusion to external solvent medium (Shi et al., 2005).

**Initial stage**

Addition of solvent to the sample takes place. The solvent runs through the cavities and capillarities of the sample by osmotic forces and fills the plant matrix while dissolving phenolic compounds so that inner concentration increases by time and a concentration
gradient is created. Also the polyphenols that are exposed or damaged during grinding processes are washed away in this stage.

**Diffusion stage**

Dissolved polyphenols diffuses from plant matrix to the solution media. Outer concentration of phenolics in external media starts to increase. This stage is similar to the initial step of delivery of a microencapsulated drug as shown in Fig. 6.

Extraction processes can be improved by selecting proper solvent type, adjusting pH, temperature, solvent to solid ratio, sample particle size, solvent viscosity. Changing these parameters to find optimum conditions can increase the efficacy of the process (Moure et al., 2001).

**2.9.1. Solvent type**

The solvent used in extraction process should be inert against the polyphenols. It should not react with any bioactive compound of interest and should keep its stability throughout the process. Polar solvents are the most used solvents to remove polyphenols from water (Moure et al., 2001). Ethyl alcohol and water are the most widely employed solvents due to hygienic and abundance reasons. Since ethyl alcohol is major component of alcoholic beverages, its presence in final product is also safe for consumption. Although using hot water as solvent is another health-safe method, it’s not preferred for heat sensitive processes because it can cause denaturation of active compounds during the process (Moure et al., 2001; Shi et al., 2005).

**2.9.2. Solvent viscosity**

Solvents with lower viscosities tend to increase extraction rate since they can pass through capillarity of plant cells faster than any other solvent with higher viscosities and in that way diffusion of polyphenol solution from plant matrix is altered.
2.9.3. pH of extraction medium

The solubility of compounds and ions change due to pH of medium (Shi et al., 2005). That is why the pH of medium should be adjusted to suitable levels. For example maximum solubility is reached at pH 4 for polyphenols from olive rape (Moure et al., 2001).

2.9.4. Particle size

Reduction in particle size either by mechanical crushing or grinding and enzyme demolition boosts solvent extraction (Moure et al., 2001). Grinding shortens the path solvent has to travel to reach plant matrix and also increases the surface area of particles providing more alternative routes for the solvent. Also employing pectinases and cellulases for cell breakdown enhances extraction (Shi et al., 2005; Moure et al., 2001).

2.9.5. Temperature

Increasing the temperature increases the extraction rate since heat decreases viscosity of solvents while increasing solubility, diffusion coefficient of phenolic compounds being extracted and cell wall permeability by increasing the size of pores on the surface. However, increasing the temperature is limited by the nature of compounds of interest. High temperatures can cause denaturation of these compounds and all process loses its meaning that’s why the optimum temperature should be lower than decomposition temperature of the phenols those need to be extracted (Shi et al., 2005; Moure et al., 2001).

2.9.6. Solvent-solid ratio

If the solvent to solid ratio is high, polyphenol concentration in the bulk solution will be low so that a greater concentration gradient will be formed between inner and outer surface of plant material (Shi et al., 2005). Although solvent extraction is widely employed, other methods such as percolation, digestion, decoction, maceration, infusion,
hot continuous extraction (soxhlet), microwave assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction (SFE), aqueous-alcoholic extraction by fermentation, counted current extraction (CCE), phytonic extraction (by hydro-fluoro-carbon solvents) are also used. Furthermore, for aromatic plants there are also methods such as head space trapping technique (HSTT), solid phase micro extraction (SPME), protoplast extraction technique (PET), micro distillation, thermo-micro-distillation, and molecular distillation techniques.

2.10. Stability of phenolic compounds after extraction

In order to use grape and papaya seeds as source of natural and antimicrobial agents, the phenolic compounds have to be extracted and converted into a dry and relatively stable form by, for example freeze drying. Stability of the extracts during storage is therefore a significant issue. Phenolic compounds are compartmentalized in the cell matrix, but once the cell matrix is broken during the extraction of phenolic compounds, the compounds become prone to degradation. It has been shown that temperature, light (Rodriguez et al., 1994), oxygen (Waterman and Mole, 1994), enzymes (Murkovic, 2003) and pH (Friedman and Jurgens, 2000) affect stability of phenolic compounds. The changes may in turn affect the antioxidant properties of the phenolic compounds.

Phenolic compounds can be degraded through enzymatic or chemical oxidation that may occur during the extraction process. Plant tissue contain the enzyme polyphenol oxidase, which when in contact with phenolic compounds in the presence of oxygen as the plant tissue disintegrate, may catalyze the oxidation of the phenols to quinones (Murkovic, 2003). The quinones formed can subsequently undergo rapid non-enzymatic polymerization that would change the original nature of the phenolic compound, hence causing changes in its chemical properties. For instance, the disintegration of plant tissue
in potatoes releases polyphenol oxidase, which reacts with phenolic compounds in the presence of oxygen resulting in the formation of polymerized brown compounds (enzymatic browning) (Kaaber et al., 2002). The non-enzymatic oxidation of phenolic compounds would however be the more likely mechanism in grape seed since polyphenol oxidase is not important in grape seed extract.

It would appear that liquid phenolic extract and freeze-dried phenolic extracts both have good storage stability at temperature range of 4–37 °C when stored in dark, but the extract stored at 25 °C exposed to light showed degradation of polyphenolic compounds into simpler ones after storage of 7 d (Mansur and Khalil, 2007). Degradation of the phenolic compounds in the presence of light is generally accompanied by a reduction in antioxidant potency (Mansur and Khalil, 2007). Based on this observed effects of light on phenolic acids, it could also be expected that flavonoids may similarly be degraded under the influence of light.

Stability of phenolic compounds however appears to be greatly affected at temperature higher than 50 °C and may lead to decrease in antioxidant activity (Azizah et al., 1999). Rates of chemical reactions increase as temperature increases; the reduction in the antioxidant activity of phenolic extracts at these temperatures may be due to increased oxidation of phenolic compounds at the high temperatures. Handling or storing the phenolic compounds at low temperatures may therefore prevent their oxidation.

2.11. Chilled storage of fish and the effect of phenolic compounds

Fish quality is very subjective in nature and is very complex concept (Bremner, 2000) which includes nutritional, microbiological, biochemical and physiochemical attributes. Spoilage of fish is not clearly defined, but can be considered as any change that renders the product unacceptable for human consumption (Huisveld, 1996). The fish
deteriorates rapidly during post-mortem storage. Chemical deterioration and microbial spoilage are responsible for an approximately 15% loss of fish and seafood every year (Ghaly et al., 2010). The freshness of fish degrades after death due to various biochemical reactions (changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine) 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 this nutritionally rich natural resource.

Chilling is considered as the most effective method for keeping fish quality (Opara et al., 2007). The quality and freshness of marine species rapidly decline post-mortem due to variety of microbial and biochemical degradation mechanism (Pigott and Tucker, 1990). Thus, the sensory quality and nutritional value of chilled fish deteriorate as a result of different degradative pathways, such as endogenous enzymatic activity, microbial development and lipid oxidation mechanism (Olafsdottir et al., 1997). To slow the mechanism involved in quality loss, the fish species should be chilled immediately after capture using ice. Therefore fish have traditionally been cooled and stored in either flake ice or ice slurries or they have been preserved by exposure to chemical agents (Hwang and Regenstein, 1995). However, the seafood industry is always searching for new preservation strategies to extend shelf life of fish and provide consumer with fish with best quality, both at sensory and nutritional levels. Effect of Aloe vera and crown of god fruit on sensory, chemical and microbiological attributes to Indian mackerel during ice storage was reported by Winarni et al. (2012) and results of the study showed that the shelf life of Indian mackerel increased by 4 d during chilled storage. Effect of dip treatments prepared with 3% and 5% green tea (Camelia sinensis) (GTE) extracts on the ice storage characteristics of Indian mackerel (Rastriliger kanagurta) was evaluated by Shinde et al. (2012) and
results shows interesting finding of increase in shelf life by 8 d. Antioxidant activity of grape and clove bud extract as natural antioxidant on silver carp fillets during chilled storage was investigated by Shi et al. (2014) and result of the study suggests that antioxidant effect of the two extracts on protein oxidation was less pronounced than the effect on lipid oxidation. Natural extract of thyme, oregano and clove improves quality of gutted and beheaded anchovy during chilled storage as examined by Bensid et al. (2014). Modified atmospheric packaging in combination with natural extract from grape seed were used to prevent lipid and protein oxidation in chill stored beef patties (Jongberg et al., 2012).

It has been estimated that one third of the world’s food production is lost annually on account of microbial spoilage. Microorganisms associated with aquatic products usually reflect the microbial population in their environment. Winarni et al. (2012) had studied that, the microbiological attributes of the Indian mackerel treated with crown of god fruit and aloe vera during ice storage. The result showed that 1.5% crown of god fruit showed lowest bacterial increase in total plate count whereas 20% of aloe vera did not affect on bacterial growth. Li et al. (2012a) have studied that, the changes in total microflora of crucian carp treated with tea polyphenols and rosemary extract during 20 d of storage indicated that dipping of crucian carp into 0.2% aqueous solutions of tea polyphenol and rosemary extract delayed the microbial spoilage and extended shelf-life of the fish. Bensid et al. (2014) also reported the effect of icing system with thyme, oregano and clove lowers count of aerobic mesophilles and psychrotrophic bacteria in anchovy muscle during chill storage.

The biochemical composition of fish or intrinsic factors and their interrelationships with post-mortem extrinsic factors, contribute substantially to the perishability of fish as a
food commodity because they determine the initial contamination (Huss et al., 1997). Sanjuasrey et al. (2012) have reported that, the combination of organic acids icing system is beneficial for decreasing the production of amines by microbes. Increase in TVB-N and TMA levels in fish may result from deamination of free amino acids, oxidation of amines, and degradation of nucleotides by autolytic enzymes and microbial activity (Ocano-Higuera et al., 2009). Gao et al. (2014) noted that rosemary extract combined with nisin treatment reduced TVB-N formation in pompano fillet during chilled storage.

Changes in pH value can be used as an indicator for determining freshness of fishery products (Campos et al., 2005). Increase in pH value of fish muscle has undesirable effects on sensorial characteristics and consequently decreases shelf life of fish meat. Raeisi et al. (2014) investigated that changes in pH of rainbow trout treated with grape seed extract during cold storage and result of the study revealed that pH of the sample treated with grape seed extract was comparatively lower than that of untreated sample which shows that it might be due to antibacterial activity of grape seed extract. Quitral et al. (2009) had reported the effect of plant extract icing system on the pH of Chilean jack mackerel and the results of the study revealed that employment of such icing system led to lower scores of pH value. Bensid et al. (2014) documented that there is no significant difference in changes in pH with the effect of icing system with thyme, orgeno and clove extracts on the gutted and beheaded anchovy during chilled storage.

Seafood products are perishable and their sensory characteristics depend on various factors, such as packaging methods, storage methods and time. The sensory characteristics of different species are very different, whether raw or cooked. The first sensory changes of chilled fish during storage are concerned with appearance, texture and odour. Indeed, physical properties as firmness and appearance are strictly related to storage days because
of cellular flaking of autolytic and microbial changes (Fletcher and Statham, 1988). Freshly caught fish contains low levels of volatile compounds which contribute to the fresh-like odours, whereas the characteristic sour and rotten odour at the end of shelf life originates from short chain fatty acids, alcohols, sulphur compounds and amines generated by microbial activity (Olafsdottir et al., 1997). Shi et al. (2014) had documented the change in sensory characteristics of silver carp fillet treated with grape seed and clove bud extract and the results of the investigation revealed that it improves sensory characteristics of fillets by 3 d. Gao et al. (2014) had reported that treatment of rosemary extract with nisin improves the sensory characteristics of pompano fillet during chilled storage. The effect of grape seed extract on the sensory quality of chicken burgers were well documented by Sayago-Ayerdi et al. (2009) and results of the study indicates hamburgers prepared with 1, 2 and 3% grape seed extract obtained higher mark value in sensory evaluation.

2.12. Freezing and frozen storage of fish and the effect of phenolic compounds

Freezing is a general preservation method used to control or decrease biochemical changes in fish that occur during storage. Nevertheless, frozen storage does not completely inhibit chemical reactions (e.g., lipid oxidation) that lead to quality deterioration of fish. However, the quality of fish muscle will also deteriorate during frozen storage as the fish muscle is abundant in protein and unsaturated fatty acids. Thus, taking some measures to extend the shelf life of fish during cold storage is of prime importance. In this aspect several authors have documented use of polyphenolic compounds as natural preservative which could inhibit lipid oxidation and microbial spoilage. Sanchez-Alonso et al. (2008) have studied the effect of grape seed on restructured fish products during frozen storage which indicated that grape seed could be used as a natural ingredient to prevent oxidation.
in minced fish during frozen storage. Dragoev (2008) have investigated the effect of the pre-storage anti-oxidant superficial treatment with natural, synthetic antioxidants and blend, containing Rosemary, Japanese acacia extracts and sodium erythrobate on the lipid peroxidation inhibition of frozen mackerel, stored 360 d at -18 °C and showed a maximum 3.09 mg malonaldehyde / kg sample at the end of storage.

During frozen or cold storage, seafood products may develop surface drying and dehydration, which may lead to freezer burn, and may suffer from quality loss owing to oxidation or rancidity. However to avoid the surface dehydration during cold storage and increase the shelf life of fish and fish products, antioxidants and glazing are the most used methods to delay the onset of oxidation or to slow down the rate of oxidation of food (Garthwaite, 1992). Coban (2013) have studied the effect of phenolic compounds of clove, sage and thyme oils as glazing material for rainbow trout (Oncorhynchus mykiss) during frozen storage and the results of the study revealed that sage oil, in combination with glazing, has a role in inhibiting the quality loss and chemical changes especially.

During the freezing and frozen storage, lipid oxidation is one of the major causes of deterioration in the quality of fish and fish products. Oxidative deterioration of lipids can directly affect quality characteristics such as colour, flavour, texture, nutritive value and safety. Lipid oxidation inhibition by grape phenolic compounds during frozen storage of mackerel and fish lipid in model system had been well documented by Pazos et al. (2005) and results of the study suggests that an optimal combination of procyanidin, degree of polymerization and percentage galloylation may be related to the highest antioxidant efficacy of grape polyphenols in the different systems tested.

Freezing and frozen storage have largely been employed to retain the sensory and nutritional properties of fish although enzymatic and non-enzymatic rancidity is known to
develop strongly under such conditions (Erickson, 2002). During frozen storage, enzymatic and non-enzymatic rancidity highly influences the shelf life of marine products due to unsaturated lipid composition and the presence of prooxidant molecules in their muscles (Ackman, 1989; Aubourg, 2002). The effects of combination of freezing and the use of antioxidant technology on the quality of frozen sardine fillets were investigated by Ozogul et al. (2011) and the results of the study revealed that 2% of rosemary extract in combination with freezing were found to be most effective in controlling the rate of lipid oxidation. Selani et al. (2011) had reported the inhibition of lipid oxidation and biochemical changes in raw chicken and cooked chicken muscle with the effect of Isabel grape seed extract (IGE) and Niagara grape seed extract (NGE) during frozen storage of nine months and the results of the study revealed that Isabel and Niagara grape residue extracts were as effective as BHT and sodium erythorbate at preventing lipid oxidation in raw and cooked chicken meat.

During frozen storage, the micro-organisms present on the surface and the tissue of fish are generally inactivated. Thus, during frozen storage, microbial changes in fish are minimal. Although some microorganisms survive at low temperature, their activities are suppressed and bacterial numbers may be reduced when recommended temperature are maintained. The experiment of (Ozogul et al., 2011) reported that combination of antioxidant and frozen storage resulted in significant reduction of bacterial growth during frozen storage of sardine fillets.