ANTIOXIDANT EFFECTS OF DIFFERENT FRUIT PEEL EXTRACTS ON SARDINE FISH UNDER CHILLED CONDITION

Thesis submitted in part fulfillment of the requirements of the Degree of
Master of Fisheries Science in Fish Processing Technology
to the Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam.

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CERTIFICATE

This is to certify that the thesis entitled, "**Antioxidant effects of different fruit peel extracts on Sardine fish under chilled condition**," submitted in part fulfillment of the requirements of the degree of **Master of Fisheries Science in Fish Processing Technology** to the Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, is a record of bonafide research work carried out by **Ms. Priyanka Dhruw, I.D. No. U-17-TN-03-002-M-F-041** under my supervision and guidance and that no part of thesis has been submitted for the award of any other degree, diploma, fellowship or similar titles or prizes and that part of thesis has been published peer reviewed journal(s) and copy / copies appended.

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Dedicated
To My
Beloved Parents

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ABSTRACT

Title	:	Antioxidant effects of different fruit peel extracts on Sardine fish under chilled condition
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The natural antioxidants from different fruit peel (pomegranate, orange and pineapple) and their combined peel extracts was extracted using methanol and crude extracts were obtained in rotary evaporator under low pressure and low temperature. Inorder to prevent the oxidation in sardine (*Sardinella gibbosa*) muscle during chilled storage. The antioxidant substances such as total phenols and total flavonoids were determined and the potential of pomegranate peel extract (PPE), orange peel extract (OPE), pineapple peel extract (PAPE) and combined fruit peel extracts (CFPE) was assessed using in vitro assays DPPH radical scavenging activity. Total phenolic and total flavonoids contents were found to be 226 mg/g GAE, 152 mg/g GAE, 141.66 mg/g GAE, 137 mg/g GAE and 177.33 mg/g QE, 88.66 mg/g QE 189.33 mg/g QE, 111.33 mg/g QE respectively. The DPPH radical scavenging activity of PPE at 4 µg/mL concentration was 88.46%, butylated hydroxyanisole (BHA) showed 91.87% of inhibition while CFPE, PAPE and butylated hydroxytoluene (BHT) showed the radical scavenging activity at a concentration of 6 µg/mL i.e. 83.09%, 76.85%, 89.54% respectively. Whereas, OPE showed the radical scavenging activity at a

concentration of 8 µg/mL i.e. 81.53 %. Standardization of effective dose application of different fruit peel and their combined extracts for sardine was done based on the biochemical, microbiological and sensory parameters at 4±1 ˚C for 72 hours. Synthetic antioxidants like BHA and BHT were taken as standard dose was also done for comparison. After standardization the pomegranate peel extracts at 1% and 3% concentration along with were selected BHA to retard lipid oxidation in sardine during storage study 15 days at 4±1 ˚C chilled storage condition. Changes in lipid was assessed by (PV), thiobarbituric acid (TBA). Apart total volatile base nitrogen (TVB-N), total plate count (TPC), sensory evaluation. The parameters revealed that the TP3 was more effective than TP1 and TB. However, when the TP1, TP3 and TB treated samples were compared with the changes in control samples, PPE (TP1 and TP3) was found to offer protective effect to certain extent against lipid oxidation. Thus, it can be concluded that the antioxidant compounds present in the different fruit peel extracts can be used effectively as natural antioxidants for controlling the oxidative rancidity in fish and fishery products.

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LIST OF ABBREVIATIONS

µg/mL	Microgram/milli liter
µl	Micro liter
ANOVA	Analysis of variance
APHA	American Public Health Association
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
°C	Degree centigrade
CFPE	combined fruit peel extracts
cfu/g	Colony forming unit/gram
cm	Centimeters
CMFRI	Central Marine Fisheries Research Institute
DHA	Docosahexaenoic acid
DPPH	Diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Oraganization
FFA	Free fatty acid
g	Gram
GAE	Gallic acid equivalents
hr	Hour
Kg	Kilogram
kgf	Kilogram-force
LDPE	Low-density polyethylene
MDA	Malondialdehyde
meq	Milli-equivalent
mg	Milligram
mg/l	Milligram/liter
mg/ml	Milligram/milli liter

min	Minute
mL	Milli liter
mm	Milli meter
Mv	Millivolt
NDGA	Nordihydroguaiaretic acid
NHB	National Horticulture Board
NER	North east region
nm	Nano metre
OD	Optical density
OG	Octyl gallate
OPE	Orange peel extract
PAPE	Pineapple peel extract
PG	Propyl gallate
PPE	Pomegranate peel extract
ppm	Parts per million
PUFA	Poly unsaturated fatty acid
PV	Peroxide value
QE	Quarceteen acid equivalents
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tert- Butylhydroquinone
TCA	Trichloroacetic acid
TFC	Total flavonoid content
TPC	Total plate count
TPC	Total phenolic content
TVB-N	Total volatile basic nitrogen
v/v	Volume by volume
WHC	Water holding capacity
w/v	Wight by volume

I. INTRODUCTION

Fish and fishery products are important sources of vital nutrients such as proteins, vitamins and minerals in addition to favorite fatty acid composition. Marine fishes and their products are the most important sources of long-chain polyunsaturated omega-3 fatty acids, vitamin D, and are favorable with respect to both cardio-vascular diseases and fetal development (Mohanty et al., 2016) The main beneficial health effects of eating seafood are linked to the omega-3 fatty acids. These long-chain polyunsaturated fatty acids are key building blocks in the cell membranes of fish, shellfish and marine mammals. Marine algae have an effective formation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), while plants and land animals generally have poor production of these fatty acids. Humans and most land animals have a limited ability to transform other fatty acids to EPA and DHA. It is therefore, important to consume sufficient quantities of these fatty acids through diet (Swanson et al., 2012).

The world fish production in 2016 was 170.9 million tons, out of which 151.2 million tons, was used for human consumption and remaining is used for non-food purpose and discarded as waste material (FAO, 2018). Many species of fish and invertebrates are rarely used as food since they are small in size and low value with a large proportion of bones and skin. Such undersized low value fishes can be utilized with value addition for income generation.

In recent years, there is an increasing demand for fish and fishery products worldwide due to the awareness on the nutritional values and health

benefits of seafood‘s. India is the second largest producer of marine fisheries mainly Sardine and Mackerel (Sinha et al., 2017).

The annual marine fish production in India was 3.83 million metric tonnes during the year of 2017 (CMFRI, 2018). Tamil Nadu shared 6.55 lakh tones in overall total marine fish production in 2017 (CMFRI, 2018). Among the total fish production in Tamil Nadu pelagic fish contributes 51.9% and demersal fish contribute 32% and 6.5% and 9.6 % of crustacean and Molluscan. Fishes like sardines form 51.9% in total pelagic resource and the major contribution of other sardines (lesser sardines) was 16.9 % and oil sardine (*Sardinella longiceps*) was 7.9 % in total fish production.

Sardines are the major landing species in Tamil Nadu coast especially in Gulf of manner region. There are about 240 species contributing to the sardine fishery and mainly nine species occurring in Indian waters. In the order of abundance, are *Sardinella gibbosa*, *Sardinella aurita, Sardina pilchardus, sardinella longiceps, sardinella albella, sardinella sirm, sardinella fimbriata, sardinella melanura* and *sardinella joneses.* They collectively contribute to a major share of the marine pelagic fisheries resources of India.

The demand for high quality fish and fishery products is growing significantly every year mostly due to their nutritional fact that they contain (FAO, 2008). The most important of these are fish lipids, which usually contains high amount of omega-3 fatty acids, mainly α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The omega-3 fatty acids have several beneficial impacts on human health. These include decreasing the risk of myocardial infarction (Bucher et al., 2002) lowering blood pressure and

triglyceride concentration in blood (Harris et al., 1997) enhancing the immune system (Damsgaard et al., 2007) and sustaining proper brain function in human body. They also protect against various psychological disorders, depression and attention deficit hyperactivity disorder in particular (Sinn, 2007) and cancer (Caygill and Hill, 1995). Nevertheless, fatty acids are not the only important nutrients in the fish and fishery products, it is also a good source of easily digestible protein, and its amino acid profile usually contains most of the essential amino acids which is required to humans for balanced diet. They are also rich source of fat-soluble and B-group vitamins (Erkan et al., 2009).

However, it is a well known fact that a huge quantity of fish is lost every year due to quality deterioration because of post-harvest fish handling as well as lack of preservation techniques that otherwise might have guaranteed the high quality of the fish product. There are three main causes of spoilage of fish and fishery products *viz.* autolysis, microbiological spoilage, and lipid oxidation. Sardine is generally considered to be high fatty fish and has vital nutrients. It is highly perishable and gets spoiled quickly due to its high level of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Chitra Som and Radhakrishnan, 2011).

Autolysis in fishes is usually due to improper handling or processing immediately after harvest and is caused by intrinsic enzymes that occur naturally in the body of fishes. The typical effects of autolysis in fishes are softening of tissue, belly bursting, and production of hypoxanthine and lactic acid, which changes the pH of seafood (Ghaly et al., 2010). Microbiological spoilage is caused mainly by microorganisms present in the fish and environment. The most important bacterial group involve in spoilage of fish and fishery products are

Pseudomonas spp., Aeromonas spp., Vibrio spp. and *Enterobacteriaceae*, which are usually present in the iced freshwater fish, and *Shewanella* species *i.e. S. putrafaciens*, *S. algae* and *S. baltica*. They are commonly found in iced marine fish (Kulawik et al., 2012). Third type of quality losses occur due to lipid oxidation. The fish and seafood contain high contents of polyunsaturated fatty acid mainly omega-3 fatty acids because of which, fish and fishery products are highly prone to lipid oxidation that causes deterioration and reduction of shelf life. Lipid oxidation may produce changes in meat quality parameters such as colour, flavour, odour, texture and even nutritional value (Fernandez et al., 1997). The development of off-flavour and rancidity in these products are the main stumbling-block in their production and commercialization (Frankel, 2005). It leads to the formation of several other compounds like primary and secondary oxidation products which have negative effects on the quality of meat and meat products causing changes in sensory and nutritional quality (Karakaya et al., 2011).

Lipid oxidation can be minimized or inhibited by the use of antioxidants in the fish and fishery products and therefore the product quality and storage stability can be improved. The antioxidants can be of synthetic or natural in origin. Synthetic antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Tert- Butylhydroquinone (TBHQ), and Propyl gallate (PG) have been widely used in food processing industry (Biswas et al., 2004; Jayathilakan et al., 2007). The above have advantages among the food producers mainly because of their lower production cost and higher antioxidant capabilities when compared to natural antioxidants. In recent years several studies have reported that, the high intake of synthetic antioxidants can be

hazardous for human health (Vandongen et al., 1993). Procarcinogenic effect demonstrated that some synthetic antioxidants such as BHA have been implicated in stomach cancer and urinary bladder cancer (Ito et al., 1986). So the demand for natural antioxidants, especially of plant origin has increased in recent years.

The natural antioxidants or antimicrobials from plants, in the form of extracts, have been obtained from different sources like fruit peel such as pomegranate, grapes, kinnow and vegetables such as broccoli, potato, drumstick, pumpkin, curry and nettle, herbs and spices tea, rosemary, oregano, cinnamon, sage, thyme, mint, ginger, and clove. These plant materials are, well investigated to decrease the lipid oxidation by several authors (Devatkal et al., 2010; Huang et al., 2011).

Pomegranate (*Punica granatum*) is a native of Iran and cultivated in various countries like Northern India and Mediterranean and South Asian region. The best growth of Pomegranate is found in the semi-arid, mild-temperate to subtropical climates, where the air is dry, summers are hot and winters are cool, such as in Afghanistan, Iran, India, China, Japan, California, Spain, Egypt, Turkey, Greece and Russia (Akbarpour et al., 2009; Ozgen et al., 2008). India is one of the leading countries in pomegranate production 2,442,000 in million tonnes (NHB, 2017). Since ancient time, various parts of the plant have been used for medicinal purposes for human health. Several authors have reported that pomegranate is used in Ayurvedic and Unani system of medicine as a therapeutic agent for the treatment of inflammatory diseases and disorders of the digestive tract (Lansky and Newman, 2007). The industrial transformation of fruits and vegetables generates large quantities of fruit waste, which has become an

environmental problem. They are rich source of bioactive compounds and may be suitable for several purposes (Viuda- Martos et al., 2009).

Oranges (*Citrus sinensis*) belong to the family ―Rutaceae‖. It is cultivated in many parts of the world like India, UK, France etc. In Asia, oranges originated thousands of years ago, in the region from southern China to Indonesia from which they were spread to India (Arora and Parminder., 2003). The orange production in India is 3,497,000 million tonnes in (NHB, 2017). It is one of the commercial fruit crops grown in the entire world. Very large amounts of by-product are formed as wastes during the production of citrus juices (Manthey and Gorhmann, 2001). Citrus by-products such as peels, seeds are useful sources of phenolic compounds and flavone glucosides mainly consisting of naringin, hesperidin, narirotin, and neohesperidin. Nowadays, citrus peels are used as the main source for natural antioxidants to be used in foodstuff for preventing rancidity, fostering stability, and inhibiting lipid oxidation, so it has attracted the attention of researchers (Anagnostopoulou et al., 2006; Peschel et al., 2006; Rahman et al., 2006). Phenolic compounds are the plant metabolites that are distributed throughout the plant kingdom and contribute to their antioxidant capacity. These compounds include phenolic acids, flavonoids, and tocopherols (Wang et al., 1996).

Pineapple is one of the most important tropical fruit grown in the north – eastern part of India. India is the fifth largest producer of pineapple and the production is 203,800 million tonnes in (NHB, 2017). The ministry of commerce and industry has recently sanctioned the agri-export zone scheme for the entire North East region (NER) at Tripura. Under this scheme, enhanced international market access would be provided to farmers besides necessary infrastructure,

flow of credit, transport assistance and other facilities for promoting agricultural export through pineapple cultivation (Saloni et al., 2017). *Ananas comosus (L.)* Merrill belongs to the Bromeliaceae family and it is an important tropical and subtropical plant widely cultivated in the tropical areas of the world. Its fruit is consumed fresh or canned as a commercial product in many countries (Avallone et al., 2003). Pineapples are rich in antioxidants namely flavonoids, vitamin A and C (Wood, 1988). Pineapple has also been known for a number of beneficial biological activities such as antioxidative, anti-browning, anti inflammatory and anti-platelet activities. The enzyme complex of *A. comosus* called bromelain is known for its clinical applications particularly modulation of tumor growth, blood coagulation and anti-inflammatory effect (Chaisakdanugull et al., 2007). Pineapple has been extensively used in foods or for health benefits. Pineapple contains fibrinolytic, antiedematous, antithrombotic, and anti-inflammatory activities.

Various synthetic antioxidants can be used in order to prevent oxidative reactions in fish and fishery products. However, because of consumer concern about the potential health hazards associated with dietary intake of synthetic antioxidants, the focus of this study is to employ natural antioxidants extracted from the peels of fruits such as pomegranate, orange and pineapple. The present investigation is focused on the ―Effect of pineapple, orange, pomegranate and their combined peel extracts on the storage stability of Sardine fish‖ under chilled condition with the following objectives

1. To investigate the antioxidant effects of fruit peel extracts on Sardine fish during chilled condition.

2. To study storage stability of fruit peel extracts treated Sardine fish under chilled condition.

II. REVIEW OF LITERATURE

There has been a growing demand of fish and fishery products due to their perceived health benefits to human. These products are highly nutritious food and provide a wide range of health-promoting compounds. Due to high content of unsaturated fatty acid, they are highly perishable especially due to microbial and biochemical spoilage compared to other food products. So special attention should be paid from the time of catch to the time of food preparation and consumption. These microbial and biochemical spoilage could be minimized by using the plant extract or low temperature storage or by using other type of preservation methods like salting, drying, smoking etc.

The nutritional relevance of sardine fish is well documented and fatty fishes in particular are of high dietary importance on account of their richness in essential omega-3 polyunsaturated fatty acids such as EPA and DHA. Inclusion of these fatty acids in diet can have potential human health benefits by prevention of many chronic lifestyle diseases (Lee and Lip, 2003). Though loaded with these benefits, a major constraint associated with the utilisation of fatty fish is their high sensitivity to oxidation resulting in numerous quality problems like undesirable odour, taste and colour in product (Frankel, 2005) as well as the health risk associated with the consumption of rancid fish. The most common method adopted for overcoming this problem is the use of antioxidant which can retard or reduce such quality changes. Natural antioxidants are getting wider publicity and consumer acceptance than the synthetic counterparts due to the toxicity and health risks associated with the latter (Anbudhasan et al., 2014).

The present review covers the occurrence of lipid oxidation in seafood, mechanisms, antioxidant activity of plant extracts. The compounds related to antioxidant properties discussed with application in fish and fishery products for enhancing their shelf- life. This review will also cover the latest finding related to application of plant extracts in enhancing the shelf life of fish and fishery products.

2.1. Lipid oxidation

Fish lipids are good and natural sources of polyunsaturated n-3 fatty acids (PUFA) such as docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3). These fatty acids have beneficial health effects and are reported to prevent coronary heart diseases and have a positive effect on the brain and nervous system as well as stimulating the immune system (Narayan et al., 2006). However, due to high content of long-chain PUFA, fish lipids are highly susceptible to oxidation. Lipid oxidation is the most important factor limiting the shelf life of seafood. The components released from these lipid oxidation have negative impacts on sensory quality of the fish products.

Food lipids are highly susceptible to oxidation processes, therefore oxidation reactions are one of the major sources of deterioration that occurs during manufacturing of food, storage, distribution and final preparation of foods. Lipids are susceptible to oxidative processes in the presence of catalysts such as heat, light, enzymes, metals, metalloproteinase and microorganisms, giving rise to the development of off-flavours in foods and loss of essential amino acids and fat-soluble vitamins (Shahidi and Zhong, 2010a).

2.1.2. Kinetics and products of lipid oxidation

The lipid oxidation process generally shows a lag phase followed by an exponential increase in oxidation rate. During the lag phase the rate of oxidation is relatively slow. When the oxidation process reaches to exponential phase, in this fatty acid decomposition products quickly form (Fennema, et al., 2007). Hydroperoxides are the main primary oxidation products, accumulating during the initiation and propagation step of the oxidation process. The time to reach maximum level of hydroperoxides in the oxidation process is related to degree of saturation, and occurs 32 earliest in highly unsaturated lipids because their hydroperoxides decompose more easily. After the maximum hydroperoxide level has been reached, a drop in hydroperoxides will theoretically be seen as the hydroperoxides decompose into a variety of secondary products (Frankel, 2005). The drop in hydroperoxide concentration is observed when the rate of decomposition into secondary products exceeds the formation rate. In theory this means that the primary oxidation products will dominate in the early stage and secondary oxidation products will dominate in later stages of the oxidation process.

2.1.3. Factors influencing the rate of lipid oxidation

There are several factors which can affect the oxidation of lipid like temperature, fatty acid composition, antioxidants, metals, Enzyme-catalysed reactions and water.

2.1.3.1. Effect of temperature

An increase in temperature causes a very strong reduction in the length of the induction period. In principle the rate of oxidation increases exponentially with an increase in temperature but the effect of temperature is complicated by

a reduction in oxygen solubility in liquids at increased temperature and by changes in partitioning of antioxidants between phases if more than one phase is present. Commonly, the rate-limiting step in the auto-oxidation pathway may change with an increase in temperature (Shahidi and Spurvey, 1996). The formation of auto-oxidation products during the induction period is slow at low temperature (Velasco and Dobarganes, 2002).

2.1.3.2. Effect of fatty acid composition

Abstraction of hydrogen atom in the propagation phase of autoxidation takes place preferentially at carbon atoms where the bond dissociation energy is low. Saturated fatty acids are very stable and do not oxidise at a significant rate. The rate of oxidation is much faster when poly-unsaturated fatty acids are present in the food (Parker et al., 2003). The relative rate of oxidation of oleic acid (18:1) and linoleic acid (18:2) has been reported to be between 1:12 and 1:40 from different studies in the literature, but the increase in rate with additional double bonds in the fatty acid is normally roughly in proportion to the number of methylene groups between pairs of double bonds. Thus, the relative rate of oxidation of 18:2, 18:3 and 20:4 is roughly 1:2:3. As well as increasing the rate of oxidation, the polyunsaturated fatty acids present in a food will produce different volatiles commonly, it is found that fatty acids with an n-3 structure such as linolenic acid produce volatiles on oxidation that are perceived as off-flavours at significantly lower levels than volatiles from an n-6 fatty acid such as linoleic acid (Choe and Min, 2005).

2.1.3.3. Effect of Antioxidants

Antioxidants may act by various mechanisms. The scavenging of lipid free radicals to produce less reactive species, and hence to interrupt the

propagation stage of lipid autoxidation, is the main antioxidant mechanism by which phenolic antioxidants such as alpha tocopherol act. However, metal chelating by antioxidants such as citric acid is also an effective mechanism of antioxidant action. Reducing compounds such as vitamin C also contribute to the total antioxidant potential of a food (Kasote et al., 2015).

2.1.3.4. Effect of metals

Metals such as iron or copper are very effective pro-oxidants even if present at ppm or less. Metals are particularly effective for catalysing the decomposition of hydroperoxides by mechanisms involving one electron transfer (Valko et al., 2005).

2.1.3.5. Enzyme-catalyzed reactions

Lipoxygenase is present in plant tissues including those of soybean, pea and tomato, as well as fish and animal. The enzyme catalyses the reaction between polyunsaturated fatty acids and oxygen to produce hydroperoxides, and other enzymes within the plant or animal tissue may contribute to the formation of volatiles from the hydroperoxides during storage. Lipoxygenase can be denatured by heating to extend the shelf-life of fish or plant foods (Baysal and Demirdovn, 2007).

2.1.3.6. Effect of water

The concentration required for a volatile component to be detected as a contributor to the flavour depends on the medium. Normally non-polar components have a higher flavour threshold in non-polar media such as edible oils than in water. The phases present in the food will also affect the rate of oxidation by affecting the activity of the antioxidants present, and by partitioning of the antioxidants between oil and aqueous phases. The term polar paradox

has been applied to the phenomenon whereby polar antioxidants are most effective in oils, whereas non-polar antioxidants are more effective in emulsions. Normally, metal chelating is less effective as an antioxidant mechanism in water- containing foods than in oils (Choe and Min, 2006).

2.2. Antioxidants

Antioxidants are defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1996). In biological systems there are various biochemical defence mechanisms, including enzymatic systems and non enzymatic antioxidants, that protect the cellular components from oxidative damage. For food systems, antioxidants are molecules that can protect macromolecules from being oxidized (Decker, 2002). Antioxidants, as a tradition, are divided into two groups as; primary and secondary antioxidants.

2.2.1. Primary antioxidants

The primary antioxidants are chain-breaking antioxidants that disrupt the oxidative free radical chain reaction by donating electrons or hydrogen atoms from the phenolic hydroxyl groups and, therefore, stabilize lipid free radicals. As a result, they inhibit or slow down the initiation phase and disrupt the propagation stage of auto-oxidation. As they act as hydrogen donors they are able to scavenge lipid radicals.

$$ROO + AH \longrightarrow ROOH + A$$

Antioxidant radicals are stable due to delocalization of the unpaired electron around a phenol ring and they cannot easily react with fatty acids. They are able to terminate radical chain process by reacting with radicals.

$$ROO\bullet + A\bullet \longrightarrow ROOA$$

The most important primary antioxidants are- BHT, BHA, α- tocopherols and PG. (Gupta, 2015).

2.2.2. Secondary antioxidants

The secondary antioxidants are just opposite to the primary antioxidants; they do not break free radical chain but are able to act through various mechanisms, such as: reducers and chelators of metals ion. These secondary antioxidants prevent or retard the lipid oxidation by suppressing the oxidation promoters, including metal ions, singlet oxygen, pro-oxidative enzymes and other oxidants. Reducing agents can reduce lipid peroxides and related oxidants through redox reactions and are also referred to as oxygen scavengers. Some secondary antioxidants such as ascorbic acid can regenerate primary antioxidants by replenishing hydrogen atom, thus inhibiting depletion of important primary antioxidants. Other secondary antioxidants promote decomposition of hydroperoxides into non-radical species, or absorb UV radiation thus protecting lipids from UV-induced photooxidation (Antonio, 2014). The main secondary antioxidants are used in food products are citric acid, phosphoric acid, EDTA, ascorbic acid, ascorbyl palmitate and sulphites (Empson et al., 1991).

There are a huge number of compounds that have been proposed to possess antioxidant activity, but only a few can be used in food products due to their adverse effects in the food. The use of antioxidants in food products is controlled by regulatory laws of a country or international standards (Karre et al., 2013). The antioxidants can be of synthetic or natural origin.

2.2.3. Synthetic antioxidants

Synthetic antioxidants are chemically synthesized petroleum based antioxidants used mainly to ―retard lipid oxidation‖ in order to preserve food stabilize the oil. The most important antioxidant compounds used in food processing are butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), octyl gallate (OG), propyl gallate (PG), tert- butylhydroquinone (TBHQ), and nordihydroguaiaretic acid (NDGA). The advantages of synthetic antioxidants, which them so popular among the food producers, they have mainly lower production cost and higher antioxidant activity when compared to natural antioxidants. On the other hand, pure antioxidants derived from natural spices are likely to have more powerful scavenging abilities, such as rosmarinic acid, which has twice the antioxidant capacity of PG (Hossain et al., 2008). Synthetic antioxidants are also less polar than their natural equivalents and therefore more soluble in lipids.

Several studies have shown that a high intake of synthetic antioxidants can be hazardous to the human health as it causes several diseases. Ito et al., (1986) reported that the pro carcinogenic effect of some synthetic antioxidants on the human health. Butylated hydroxyl toluene has been linked to urinary bladder cancer and thyroid cancer. Groten et al. (2000) reported that BHT, when applied together with PG, caused joint pathology and liver enlargement. They recommended that the levels of PG and BHT in foods should be limited when both are present in the same product.

2.2.4. Natural antioxidants

Natural antioxidants are widely distributed in plant materials, animal tissues and microorganisms; they are protecting them from oxidative stress.

They can be isolated as pure compounds from source materials and can be used for food preservation. Higher plants and their constituents are the richest sources of natural antioxidants. Fruits, vegetables, spices, herbs, cereals, grains, oilseeds, leguminous seeds, tea, coffee and cocoa are the major source of plant-derived antioxidants and also phenolics and carotenoids ascorbic acid and tocopherols have been widely used as the natural antioxidant (Shahidi and Zhong, 2010b).

2.2.4.1. Phenolics compound as natural antioxidants

The term ―phenolic‖ is used to define substances that possess one or more hydroxyl groups (OH) substituents bonded on to an aromatic ring. The compounds that have several or many phenolic hydroxyl substituents are called as polyphenols. Due to its chemical structure phenolic compounds have the ability of phenoxide ion delocalize. The phenoxide ion can lose a further electron to form the corresponding radical which can also delocalize by phenolics compounds. In reference to this property, phenolic compounds have radical scavenging and antioxidant activity (Waterman and Mole, 1994). Phenolics are large and heterogeneous groups of secondary plant metabolites. They are distributed throughout the plant kingdom and they have a wide variety of structures. Major phenolic compounds are flavonoids, tannins, and phenolic acids (Rababah et al., 2004). Due to health hazard of synthetic antioxidants, there has been increasing interest in searching of natural phenolic compounds in plants to minimize lipid oxidation in seafood. Mainly these types of natural antioxidants come from fruits, vegetables, spices, grains, and herbs. Hundreds of natural phenolic compounds have been reported to possess high antioxidant activity. Their use in foods, however, is limited by certain requirements not the

least of which is adequate proof of safety. Only a few of them can be commercially applied in foods. For maximum efficiency, primary antioxidants are often used in combination with other phenolic antioxidants or with various metal sequestering agents, e.g. tocopherols with citric acid and isopropyl citrate (Nawar, 1983).

2.2.5. Mechanisms of antioxidants

The main antioxidant mechanisms are free radical scavenging, chelation of metals, removal of peroxides or reactive oxygen species, and quenching of secondary lipid oxidation products that produce rancid odours (Decker, 1998).

2.2.5.1. Free radical scavenging

Radical scavengers usually donate one electron to the unpaired electron of the free radical and thus reduce it and they produce relatively stable antioxidant radicals with low standard reduction potential, less than 500 millivolt (Mv). Polyphones are very active in this respect and the radical-scavenging activities of gallates, nordihydroguairetic acid and flavonoids arise from this process. Aromatic amines inhibit the auto-oxidation via same electron-transfer mechanism. The higher stability of antioxidant radicals than that of food radicals is due to resonance delocalization throughout the phenolic ring structure (Choe and Min., 2006). The effectiveness of any antioxidants to scavenge free radicals of food depends on the bond dissociation energy between oxygen and a phenolic hydrogen, pH related to the acid dissociation constant, and reduction potential and delocalization of the antioxidant radicals (Litwinienko and Ingold. 2003).

2.2.5.2. Chelating of metals

Metal chelators decrease oxidation by preventing metal redox cycling, forming insoluble metal complexes, or providing steric hindrance between metals and food components or their oxidation intermediates (Graf et al., 1990). EDTA and citric acid are the most common metal chelators in food. Most chelators are water-soluble, but citric acid can be dissolved in oil with some limitation to chelate metals in the oil system. Phospholipids also act as metal chelators (Koidis and Boskou, 2006). Flavonoids can also bind the metal ions (Rice-Evans et al., 1995) and the activity is closely related with the structural features. Lignans, polyphones, ascorbic acid, and amino acids such as carnosine and histidine can also chelate metals (Decker, 2002). Polyphosphates are added to inactivate iron, for example, in meat products.

2.2.5.3. Inactivating lipoxygenase

Lipoxygenase is a catalytic enzyme in the oxidation of lipids and is inactivated by tempering, which is heat treatment with moisture. Glucose oxidase coupled with catalase is the best known commercially available system to remove oxygen from food (Raba et al., 1995).

2.2.5.4. Synergism

Several mechanisms are involved in synergism among antioxidants with a combination of 2 or more different free radical scavengers in which one antioxidant is regenerated by others, a sacrificial oxidation of an antioxidant to protect another antioxidant, and a combination of 2 or more antioxidants whose antioxidant mechanisms are different (Decker, 2002). Regeneration of a more effective free radical scavenger by a less effective free radical scavenger occurs mostly when one free radical scavenger has a higher reduction potential than the

other. The free radical scavenger having a higher reduction potential acts as a primary antioxidant (Shao et al., 2008).

2.3. Natural antioxidants from fruits

Natural antioxidants, particularly in fruit and vegetables have gained increasing interest among consumers and researchers because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Renaud et al., 1998). The defensive effects of natural antioxidants in fruit and vegetables are related to three major groups; vitamin, phenolics and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants (Halliwell, 1996). Fruits and Fruit juices are important dietary components which are rich in antioxidants. Synthetic antioxidants will have potential health risks. Hence an increasing attention should be paid to identify natural and possibly more economic and effective natural antioxidants.

In the recent years, more attention has been paid to the antioxidants contained in fruits. Guo et al. (2003) claimed that high fruit intakes were associated with reduced mortality and morbidity of cardiovascular disease and some types of cancer. Fruits are diverse in antioxidant composition and antioxidant activity and those with high antioxidant activity generally contain more antioxidants. Interestingly, the peel fractions of some fruits possess higher antioxidant activity than the pulp fractions. Study by Li et al. (2006) reported that pomegranate peel exhibited higher antioxidant activity compared to its pulp. The peel fractions of fruits may potentially contain more antioxidants quantitatively or qualitatively than the pulp fractions.

In fruit processing industry and in fruit shops the fruit peels are thrown or dumped as waste, but the real fact is that the peels are having better biological activities than other fractions. Fruits and vegetables wastes and their by-products formed during industrial processing possess a serious problem as they exert harmful impact on environment. So they need to be managed and utilized (Duda-Chodak and Tarko, 2007). Sawalha et al., (2009) reported that Citrus fruits have peculiar fragrance partly due to flavonoids and limonoids present in the peel and these fruits are good sources of vitamin C and flavonoids. Many studies have reported on the antioxidant and antibacterial effect of juice and edible parts of oranges of different origin and from different varieties (Rapisarda et al. 1999; Farag et al., 1989).

Fruit peel extracts from oranges were found to have a good total radical antioxidative potential (Gorinstein et al., 2001). High content of phenolic compounds and antioxidant activity of apple peels were found to have valuable source of antioxidants and can impart health benefits when consumed (Wolfe et al., 2003). Gorinstein et al. (2001) found that the total phenolic compounds in the peels of lemons, oranges, and grapefruits were 15% higher than that of the pulp of these fruits.Peels from apples, peaches, pears as well as yellow and white flesh nectarines were found to contain twice the amount of total phenolic compounds as that contained in fruit pulp. Apple peels were found to contain up to 3300 mg/100 g of dry weight of phenolic compounds.Total phenolic compound of seeds of several fruits, such as mangos, longans, avocados, and jackfruits, were higher than that of the edible product, Grape seeds and skins, the byproducts of grape juice and white wine production, are also sources of several

phenolic compounds, particularly mono, oligo, and polymeric proanthocyanidins (Shimon et al., 2012).

2.4. Pomegranate peel as a sources of natural antioxidants agents

Pomegranate (*Punica granatum*) is native from Iran to northern India and cultivated over the whole Mediterranean region. It is an ancient, mystical, unique fruit that is widely cultivated in parts of Asia, North Africa, the Mediterranean and the Middle East (Sarkhosh et al*.,* 2007). Pomegranate peel and seed is by-product obtained during processing of pomegranate juice. The edible part of pomegranate contains the higher amount of acids (ascorbic acid, citric acid, and malic acid), sugars, vitamins, polysaccharides, polyphones and important minerals (Maskan, 2006).

Pomegranate peel contains substantial amount of polyphenols such as ellagic tannins, ellagic acid and gallic acid. Gil et al. (2000) reported that the antioxidant activity shown by the pomegranate peel extract may be due to the presence of polyphones, such as ellagic tannins, ellagic acid and gallic acid. Ozkal and Dinc. (1994) reported that pomegranate rind is a rich source of tannins and other phenolic compounds. Negi et al. (2003) recorded the significant relation between phenolic content and anti-oxidant effect of pomegranate peel extract. The health benefit properties of pomegranate peel was due to presence of wide range of photochemical, especially polyphenols, like ellagitannins, and anthocyanins (Martos et al., 2010). These polyphenols exhibit various biological activities, such as eliminating free radicals, inhibiting oxidation and microbial growth, and decreasing the risk of cardio and cerebrovascular diseases and some cancers (Caliskan and Bayazit, 2012).

2.5. Orange peel as source of natural phenolic compounds

Orange (*Citrus sinensis*) belong to the family Rutaceae. Orange constitutes about 60% of the total citrus world production (Hegazy and Ibrahium, 2012). Orange peels represent between 30 to 40% of total weight of the fruits and remain as the primary by- product of the fruit industry, If not processed it causes odor, harbourage for insects and can give rise to environmental pollution (Mandalari et al., 2006). Citrus fruits peel and juices are very important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human health (Jayaprakasha et al., 2003).

The flavanones, flavones and flavonols are three types of flavonoids that occur mainly in citrus fruit (Calabro et al., 2004). Schieber et al. (2001) reported that the flavonoids found in citrus species are hesperidins, narirutine, naringin and eriocitrin. Peterson and Dwyer (1998) classified the flavonoids found in citrus into six categories based on their structure: flavones, flavonons, flavanons, flavoenols, isoflavones and anthocyanidines. Flavonoids demonstrate their antioxidant activities in three ways: by neutralizing free radicals, by reducing the concentration of local free radicals and by chelating metals (Bombardelli and Morazzoni, 1993). Research has revealed that the antioxidant activities of citrus depend on their total phenolic compounds content (Moure et al., 2001).

2.6. Pineapple peel as source of natural phenolic compounds

Pineapple (Ananas comosus, Bromelaceae) is one of the most consumed tropical fruits and its consumption has been related to several beneficial properties such as antioxidant (Hossain and Rahman, 2011). Study

has shown that pineapples contain phenolic compounds, namely the quercetin, flavones-3-ol, flavones (Mhatre et al., 2009), p-coumaric acid and ferulic acid (Hui, 2007), and vitamin C (Kongsuwan., 2009), anti-inflammatory (Hale et al., 2005) and anti-diabetic activities (Xie et al., 2006; Riya., 2013). Correia et al. (2004) established a relationship between antioxidant activity, beta glucosidase and total phenolic content in pineapple peel/ soy flour extract. Pineapple fruit is considered a highly nutritious fruit because it contains a high level of vitamin C, a natural antioxidant which may inhibit the development of major clinical conditions including heart disease and certain cancers. The fruit also contains phenolic compounds and β-carotene, which constitute natural sources of antioxidants. Pineapple fruits are an excellent source of vitamins and minerals. One healthy ripe pineapple fruit can supply of about 16.2% of daily requirement for vitamin C. Several physiochemical parameters like starch, reducing sugar, non reducing sugar, total sugar, protein, ascorbic acid are present in juice and waste.

2.7. Extraction of natural antioxidants compounds from plants

2.7.1. Methods of extraction and solvent used for extraction

The main aim of the extraction process is to provide the maximum yield of the antioxidants and antimicrobial substance with the higher antioxidant and antimicrobial activity even at low concentration. There are lot of techniques available for extraction antioxidants from plants, such as Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound assisted extraction etc. Generally, the plant material is cleaned, dried, and ground into fine powder followed by solvent extraction. Different solvents, either separately or in combination have been used for extraction process. These solvent systems included absolute ethanol, 90% ethanol, 80%

ethanol, 70% ethanol, acetone, methanol, dimethyl sulfoxide, hexane, and water.

The presence of various antioxidant and antimicrobial compounds with different chemical nature and polarities may or may not be soluble in a particular solvent (Turkmen et al., 2006). Mainly polar solvents are often used for recovering polyphenols from plant materials. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate (Do et al., 2014). Ethanol has been known as a good solvent for polyphenol extraction. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols, whereas aqueous acetone is good for extraction of higher molecular weight flavanols (Dai and Mumper, 2010). Pre-treatment of the sample, solvent–sample ratio, type of solvent, temperature and time of extraction are the main factors affecting the extraction process efficiency (Casazza et al., 2012). A large number of studies have showed that extraction method can alter the antioxidant activity and total phenolic content. The extraction method must enable complete extraction of the compounds of interest and must avoid their chemical modification (Zuo et al., 2002).

Generally, organic solvents are very effective for extraction of antioxidants but they can affect human health if residues left in the final product which will not be acceptable for consumers. So, extra care must be taken to remove all the traces of solvent. Water is the safest solvent, but less efficient in extracting all the antioxidants. Also, the processing methods affect the nature of the extract. Therefore, the use of safer alternative solvents and methods of extraction needs to be further examined for extraction processes. In addition, the cost-effectiveness of the extraction processes needs to be monitored to

minimize the cost of natural antioxidants and ensure their wider utilization in the food industry (Vuong et al., 2011).

2.8. Studies on efficacy of plant extracts and its effect on quality of food items

Hegazy and Ibrahium (2012) evaluated the antioxidant activities of Orange peel extracts extracted from different solvents, and they revealed that all extracts of the orange peel exhibited variable antioxidant activity. Specially, the ethanolic extract showed the highest ($p<0.05$) values for yield (%), TPC, TFC, chelating and antioxidant activities % DPPH scavenging activity.

Sofi et al. (2016) studied the antioxidant and antimicrobial properties of grape and papaya seed extracts and their application on the preservation of Indian mackerel during ice storage and they reported that the grape seed extracts was most effective compared to papaya seed for controlling the lipid oxidation and microbial spoilage of Indian mackerel during ice storage.

Kanatt et al. (2009) studied the antioxidant and antimicrobial activity of pomegranate peel and seed extract and they reported that the, pomegranate peel extract showed excellent antioxidant activity while the seed extract did not have any significant activity. The IC_{50} value of pomegranate peel for DPPH radical scavenging was 4.9µg/ml while that of BHT was 21.2 µg/ml and the efficacy of pomegranate peel extract in scavenging hydroxyl, reducing power and iron cheating capacity and superoxide anion radical was also very high. In addition this had good reducing power and iron chelation capacity. Addition of pomegranate peel extract to popular chicken meat products enhanced its shelf life by 2–3 weeks during chilled storage.

Dsouza and Skonberg (2011) evaluated the antioxidant properties of

aqueous and methanol soy extracts in minced trout muscle and they found that, freeze-dried extracts were applied (1000 and 4000 mg/g) to trout mince and TBARS formation monitored during 14 days refrigerated storage. Soybean meal aqueous extracts-treated mince (4000 mg/g) had significantly lower ($p<0.05$) TBARS than all other treatments. Soybean meal extracts were most effective at retarding lipid oxidation of refrigerated trout mince.

Tang et al. (2001) evaluated the anti-oxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle and they found susceptibility of untreated minced muscle to lipid oxidation was in the increasing order: mackerel > beef > duck > ostrich > pork > chicken > whiting. The TC300 significantly ($p < 0.05$) reduced lipid oxidation compared with controls for all seven species as shown by lower TBARS values. The anti-oxidant potential of catechins was two to four folds greater than that of α-tocopherol at the same concentration and this potential was species dependent. The results revealed that tea catechins are powerful natural antioxidants when used in minced muscle food.

Cadun et al. (2008) investigated the effect of the antioxidant activity of rosemary extract on marinated deepwater pink shrimp (*Parapenaeus longirostris*) stored at 1 ^{o}C, and found that rosemary extract addition prolong the shelf-life of marinated shrimp.

Ozyurt et al. (2010) investigated the capability of the rosemary extract on the oxidative stability of cooked sea bream during frozen storage and they reported that the considerable increase in FFA, PV and TBA were observed in all cooked groups during frozen storage period. The treated samples with rosemary extract generally showed slower PV and TBA formation than those of

the untreated samples. However, the additions of rosemary extract have positive effect on sensory quality of baked sea bream.

Dembele et al. (2010) investigated the effects of added green tea polyphenol on the lipid oxidation of common carp and catfish during refrigerated storage, carp minced fillet containing 0.03% green tea polyphenol showed the best characteristics (p<0.05) in relation to oxidative stability. For catfish, 0.05% green tea polyphenol concentration was more effective in delaying lipid oxidation, when compared with the control sample and standards vitamin *C*. Further, 0.01%-treated minced fillet with vitamin C was less effective than 0.01% green tea polyphenol in reducing lipid oxidation.

Erkan and Bilen. (2010) studied the effect of essential oils treatment on the frozen storage stability of chub mackerel fillets and revealed that the sensory attributes like taste, odour, texture and overall acceptability of control samples were given ‗unacceptable' scores by the sixth month. Based on sensory examination the shelf-life of frozen chub mackerel were found to be 6 months for samples treated with oil of thyme, rosemary, black seed, sage and lemon and 7 months for samples treated with bay leaf, grape seed and flaxseed. TBA and FFA values for all treatments remained lower than control samples throughout the 11 months storage period. Thyme oil treatment was effective in delaying lipid oxidation compared to other extracts.

Iglesias et al. (2009) studied the caffeic acid as antioxidant in fish muscle, mechanism of synergism with endogenous ascorbic acid and α-tocopherol and they reported that the, addition of caffeic acid to fish muscle retarded both the degradation of endogenous α-tocopherol and the propagation of lipid oxidation, measured by PV and TBARS, with increasing effect upon

increasing the addition of caffeic (55.5-555.1 *μ*mol/kg).These results indicate that the antioxidant mechanism of caffeic acid implies the protection of endogenous α-tocopherol localized in tissue membranes where lipid oxidation is initiated and, at the same time, caffeic acid regeneration by the endogenous ascorbate. These combined effects result in a stronger antioxidant protection against lipid oxidation by favoring, as a final point, the protection of α-tocopherol, which is suggested as the last defence of fish muscle against lipid oxidation.

Erkan et al. (2011) studied the effect of combined application of plant extract and vacuum packaged treatment on the quality of hot smoked rainbow trout and they reported that the sample stored without any treatment control group was spoiled after four weeks of storage. In the sample treated with plant extracts decreased microbiological activity. Shelf life of hot smoked trout stored in cold storage (-18±2 ^{o}C), was observed as 6 weeks for rosemary, black cumin seed, and lemon oil treatment plus vacuum packaged fish and 7 weeks for bay leaf oil treatment plus vacuum packaged smoked fish.

Houicher et al. (2015) studied the effect of natural extracts *mentha spicata* and *Artemisia campestris* on biogenic amine formation of sardine vacuum-packed and refrigerated fillets and they reported that natural extract treatments combined with vacuum packaging resulted in a significant shelf life extension of the sardine fillets for 7 days. Treated groups had lower (P<0.05) histamine, tyramine and cadaverine contents than control samples. Mint extract was most effective in decreasing biogenic amine production in fish muscle, while the artemisia extract treatment contributed to maintain histamine- forming bacteria at low levels until day 17 of chilled storage. The findings suggested

that the application of mint and artemisia extracts improve the food safety and increase product‘s shelf life without altering the sensory properties of fish.

Pezeshk et al. (2012) studied the evaluation of shelf life of live and gutted fish treated with a shallot extract and they reported that the immersion of fish in shallot extract increased shelf life of fish stored at refrigeration temperatures. It can improve the quality of food products during refrigerated storage because of the presence of sulfur-containing compounds and polyphenolic derivatives in its composition. Shallot extract promotes health by preventing lipid oxidation, and has been shown to possess antibacterial characteristics. In the food industry, this could be used as alternatives to the synthetic antioxidants because the use of these types of antioxidants is controlled because of concern for their carcinogenic potential.

Yuan et al. (2016) investigated the effect of chitosan coating combined with pomegranate peel extract on the quality of pacific white shrimp during iced storage. Chitosan coating combined with Pomegranate peel extracts could inhibited the melanosis and change of color difference, and improved the sensory quality, hardness and springiness of Pacific white shrimp during 10 days of iced storage. The chitosan coating combined with PPE also inhibited the increase in the TPC, pH and TVB-N and other quality parameters in pacific white shrimp.

Pal et al. (2017) studied the assessment of the shelf life of fish fillet treated with green tea extracts under refrigerated condition and they reported that the first and second extracts of green and black tea (*Camelia sinensis*) were used as an antioxidant and antimicrobial agent for *Pangasius sutchi* fillets. Phenolic compounds of tea extract resulted in microbial inhibition, protecting

fillets against the internal protease and finally inhibit protein breakdown and amine production. Under refrigerated storage lower ($p<0.05$) TBARS, PV and FFA values were recorded in all treated samples and were within the limit of acceptability till 15 days indicating a significant antioxidant effect of 1st and 2nd extracts. Higher values of protein solubility ($p<0.05$) and WHC ($p<0.05$) in treatments concluded the preservative properties of tea extract that has successfully reduced the freeze induced protein denaturation. The lower values of TPC encountered in fillet treated with tea extract ($p<0.05$) suggests that both the green tea extracts possess antimicrobial properties that resulted in growth inhibition of the bacteria. The shelf life of the fillet treated with tea extracts is adjudged 12 days for refrigerated storage.

Ozen and Soyer (2018) studied the effect of plant extracts on lipid and protein oxidation of mackerel (*Scomber scombrus*) mince during frozen storage and investigated that the effects of different plant extracts [green tea extract (GTE), grape seed extract (GSE), and pomegranate peel extract (PRE)] at a level of 100 ppm equivalent phenolics and butylated hydroxytoluene (BHT) on the changes in quality of fish (Scomber scombrus) mince during 6 months frozen storage at -18 ± 1 ˚C. During storage, significant oxidative reactions in both the lipids and proteins were observed with the increase in thiobarbituric acid reactive substances (TBARS) and carbonyls and decrease in sulphydryl groups and protein solubility. BHT and PRE effectively inhibited lipid oxidation as lower peroxide and TBARS values were observed.

Sarojini et al. (2019) studied the effect of pomegranate (*Punica granatum*) peel extract on lipid oxidation in sardine fish oil and they reported that the prospective use of pomegranate (*Punica granatum*) peel extract (PPE) as an

effective antioxidant in sardine fish oil. Total phenolic content and antioxidant activity of PPE was examined. Three different concentrations of PPE (1000, 1500 and 2000 ppm) were used to find out the appropriate concentration and also compared with the synthetic antioxidant butylated hydroxyanisole (BHA). The sardine (*Sardinella longiceps*) fish oil which was not treated with any antioxidant was maintained as control. The primary and secondary oxidation products formed in the sardine oil was determined by peroxide value and thiobarbituric acid analyses.

2.9. Shelf life of fish and fishery products

Shelf life of food is defined as the maximum length of time given to a product fit for human consumption. In case of fish shelf life is the time from when it is taken from the water until it is no longer fit to eat. In the market the shelf life of fresh and frozen fish is very important because it determines the quality of fish and fishery products. Knowing the remaining shelf life allows the processor and retailer to plan the length of time a product can be held, allowing control of their market. Temperature and handling practices are the most important factors in determining the shelf life of all species of fish. If the fish product is handled carefully, the temperature at which it is held controls its useful life. To reduce spoilage and biochemical degradation in fish and fishery products, different preservative methods are used, mainly based on low temperature, have been employed for storage and distribution of food products. The most used methods include refrigerated ice storage between 0 ^{o}C and 4 ^{o}C, superchilled storage in the range of -1 to -4 ^{o}C, by means of slurry ice or in super chilled chambers without ice, and frozen storage at -18 to -40 ^{o}C (Gallart-Jornet et al., *2007*).

III. MATERIALS AND METHODS

3.1. Materials

3.1.1. Raw materials

Fresh sardine (*Sardinella gibossa)* was procured from Tuticorin fishing harbour landing centre in fresh condition and transported to the lab by keeping it in a ice box containing fresh fish with flake ice with ratio 1:1. The 1 kg each fruit peels (pomegranate, orange and pineapple) were collected from FC & RI girl‘s hostel and different juice vendors in Tuticorin local market in fresh and ripe condition for the extraction.

3.1.2. Chemicals/ Reagents/ Glasswares

All the chemicals and reagents used in this study were obtained from Himedia and the glasswares were of Borosil made.

3.1.3. Machineries and equipments

The sardine fish was packed in low-density polyethylene (LDPE) pouches and sealed by electrical sealing machine. Electronic balance (make Citizen, India) was used for weighing purpose. Hot air oven for drying purpose and muffle furnace were used for estimation of moisture and ash contents respectively. Kjeldhal unit was used for estimation of crude protein. Soxhlet unit was used for the estimation of fat. UV-Visible Spectrophotometer was used for estimation of TBA (Thiobarbituric acid) value and estimation of phytochemicals (antioxidant, phenolic contents and flavonoid contents). Rotary evaporator was used for

extraction of fruits peel. Refrigerator was used for storage of dressed sardine. For texture analysis (TA-PLUS Texture Analyzer, Lloyd instruments, U.K.)

3.2. Methods

3.2.1. Preparation of raw materials

Fish was washed thoroughly with chilled water to remove adherent dirt. Dressing was done by descaling, deheading and again washed thoroughly with chilled water to free them from blood, viscera and mucus.

3.2.2. Preparation of fruit peel extracts

Pomegranates peel (PP) and orange peel (OP) - were procured from FC & RI girls hostel and juice vendors in the local market Tuticorin in ripe condition and peeled. The fresh peels sample was washed under running tap water and cut into small pieces and both peels were dried in hot air oven at 50 ˚C until constant weight. The dried peels were ground in the kitchen grinder to make the fine powder to pass through 1 mm sieve. The extraction was carried out according to the methods described by Yerlikaya et al. (2010). About 100 gm of peel powder was mixed with 600 ml of methanol then kept in a water bath at 40 ˚C for 4 h. The mixture of peel powder and methanol subjected to shaking at ambient temperature for 12 h at the speed of 180 rpm. The mixture were filtered with whatman paper no.1 and concentrated in a rotary evaporator at the temperature of 50 C̊ to get crude extracts. The extract was obtained from both the peels powder were weighed to calculate the yield.

Pineapple peel (PAP) - Fresh peel sample was washed under running tap water, dried in dryer at 40-50 ˚C for 72 hrs. and then homogenized to fine

powder. 100g of dried peel powder was extracted in 500 ml of methanol in rotary shaker for 10 h. at the speed of 180 rpm. Extracts were filtered in whatman paper no. 42 and concentrated in a rotary evaporator at the temperature of 50 ̊C to get crude extract. The extract was obtained from the peel powder were weighed to calculate the yield (Kalaiselvi et al. 2012).

Combined fruit peel extracts were prepared the extraction of all three fruit peel extracts (pomegranate, orange and pineapple) were mixed in equal ratio or amount (10gm each fruit peel extract).

All pomegranates, orange and pineapple peel extracts and combined fruit peel extracts were stored 4±1°C in a dark amber colored glass bottle for further analysis.

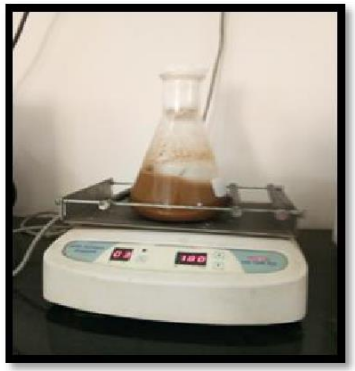
Plate 1. Preparation of fruit peel extracts





Different fruit peels (pomegranate, orange and pineapple) → Dried in hot air oven → Peel powder




Get crude extract ← Extract concentrate in rotary evaporator ← Filtration of extract ← Extracted in rotary shaker at 180 rpm ← Water bath at 40˚C for 4 hr.

3.2.3. Treatments

According to the first objective, Standardization of application of different fruit peel extracts and their combined fruit peel extracts, BHA and BHT on sardine fish based on microbiological parameter such as total plate count, biochemical parameter such as total volatile base nitrogen (TVB-N), thiobarbituric acid (TBA) , peroxide value (PV), sensory evaluation – appearance and texture analysis. Fresh sardine (*Sardinella gibossa)* was procured from Tuticorin fishing harbour landing centre in fresh condition. Dip treatment for 5 minutes given to the dressed fish in two concentrations 1 % and 3 % (Saoni, 2016) of pomegranate peel extract and a standard dose of BHA (200 ppm) using. The ratio of fish to solution was 1:1. For each experiment, total fish was divided into four batches. One batch was soaked in 1% concentration of fruit peel extract and their combined peel extracts solution, second batch in 3% concentration and other BHT and BHT 200 ppm concentration. In each experiment, one batch was kept as control (CON) without any treatment. The control and fruits peel extract treated fish were packed in LDPE pouches (120 gm per pouch) and heat sealed. In each experiment, each fruits peel extract treated batches including control were stored in a refrigerator at 4±1°C for 72 hrs. in chilled storage condition. Sampling of fish (both treatment and control) was done every day. During sampling four packets of fish from each batch were taken randomly for microbiological and biochemical analysis as well as for sensory evaluation.

According to the 2nd objective, again fresh sardine (*Sardinella gibossa)* was procured from Tuticorin fishing harbour landing centre in fresh condition. Dip treatment for 5 minutes given to the dressed fish in two concentrations (1 % & 3%) of pomegranate peel extract and a standard dose of BHA (200 ppm) using.

The ratio of fish to solution was 1:1. For each experiment, total fishes were divided into four batches. One batch was soaked in 1% concentration pomegranate peel extract solution, second batch 3% concentration and other BHA 200 ppm concentration. In each experiment, one batch was kept as control (CON) without any treatment. The control and pomegranate peel extract treated fish were packed in LDPE pouches (120 gm per pouch) with heat sealed. In each experiment, pomegranate peel extract and BHA treated batches including control were stored in a refrigerator at 4±1°C for 15 days in chilled storage condition. Sampling of fish (both treatment and control) was done at three days interval. During sampling four packets of fish from each batch were taken randomly for microbiological- Total plate count and biochemical analysis such as total volatile base nitrogen (TVB-N), thiobarbituric acid (TBA) and peroxide value (PV), as well as for sensory evaluation and texture analysis.

Treatment details	Code Name of Sample
Treated with pomegranate peel extract 1 % concentration for five min.	TP1
Treated with pomegranate peel extract 3 % concentration for five min.	TP3
Treated with orange peel extract 1% concentration for five min.	TO1
Treated with orange peel extract 3% concentration for five min.	TO3
Treated with pineapple peel extract 1% concentration for five min.	TPA1
Treated with pineapple peel extract 3 % concentration for five min.	TPA3
Treated with combined fruit peel extracts 1 % concentration for five min.	TCF1
Treated with combined fruit peel extracts 3 % concentration for five min.	TCF3
Treated with BHA for five min.	TB
Treated with BHT for five min.	TT
Without treatment	CON

Plate 2. Preparation of raw materials, treatments and analysis of biochemical, microbiological parameters

Fresh sardine brought for experiment

Solution from different fruit peel extracts

Dip treatment of sardine for 5 min.

Treated sardine packed in LDPE pouch

Treated sardine kept at 4±1˚C

Analysis of biochemical and microbiological parameters

3.3. Analysis

3.3.1. DPPH scavenging activity of different fruit peel and their combined peel extracts

The DPPH radical-scavenging activity of fruit peel extracts, were determined followed by the method of Zhang et al., 2010. Briefly, 100 µl samples at various concentrations (2% and 4% in methanol) were distributed into different test tubes and then 3.9 ml of a DPPH solution (25 mg/l methanol) was added to each tube. The mixtures were kept, in the dark, for 30 min at room temperature. Methanol was used as blank and had no DPPH scavenging activity. The decrease in DPPH absorbance (A) was measured at 517 nm. The DPPH radical-scavenging activity was calculated using the following formula:

Scavenging activity (%) = $Abs_{control}$ - Abs_{sample} / $Abs_{control}$ x 100

3.3.2. Total phenolic content of different fruit peel and their combined peel extracts

Total phenolics of fruits peel extracts were determined using the method of Singleton and Rossi, 1965 and the results were expressed as gallic acid equivalents. 200 µl portions of diluted extracts were introduced into test tubes followed by addition of 1000 µl of Folin-Cio- calteu reagent (1:10). Thirty seconds later and just prior to 8 min, 800µl of Na_2CO_3 (7.5%) was added to extracts in tubes. The reaction mixtures were incubated at 24°C for 1 h prior to recording the absorbance at 765 nm against blank. Total phenolics content of the peel extracts was calculated by using standard curve prepared from gallic acid at

concentrations range of 20 to 100 µg/mL.The total phenolic content of the sample was expressed as mg gallic acid equivalents (GAE) per g extract.

3.3.3. Total flavonoid content (TFC) of different fruit peel and their combined extracts

TFC of the extracts were determined according to the colorimetric assay following the procedure of Sultana et al., 2008. One millilitre of aqueous extract containing 0.01 g/ml of dry matter was placed in a 10 ml volumetric flask, and then 5 ml of distilled water was added. At zero time, 0.3 ml of (5% w/v) NaNO2 was added. After 5 min, 0.6 ml of (10% w/v) AlCl3 was added. After another 5 min, 2 ml of 1M solution of NaOH was added. After that, the volume was made up to 10 ml with distilled water. The mixture was shaken vigorously and the absorbance of the pink color development was measured at 510 nm against the blank using spectrophotometer. Quantification was expressed by comparing the absorbance of quarcetin calibration curve as the standard for flavonoid 20 to 100 µg/mL. Results were expressed as mg quarceteen acid equivalents (QE) per g extract.

3.3.4. Periodical sampling

For periodical biochemical and microbiological analysis, fish from pouches (one from each pouch) was minced with knife and mixed homogenously to consider as representative sample. Triplicate analysis for each representative sample was done for each determination.

3.4.5. Estimation of total volatile basic nitrogen (TVBN) (Conway. 1947)

The theory for estimating TVBN is that the released amines (smelling compounds) are first separated by precipitating the proteins using the trichloroacetic acid solution. The filtered solution (TCA extract), which contains the volatile substances, is added to a strong alkali. The volatile nitrogenous substances are distilled over and trapped by the standard H_2SO_4 solution. The remaining acid can be back titrated with the standard NaOH.

Procedure: Conway cups and lids were washed and dried. Paraffin wax and Vaseline in the ratio of 1:2 was melted and cooled. This was applied on the rims of cups. 1 ml of 0.02(N) H_2SO_4 was added into the inner chamber of each cup. Lid was placed over the Conway cup covering part of outer chamber and complete inner chamber. 1 ml of TCA extract was taken in the outer chamber followed by 1ml of K_2CO_3 solution. The unit was lidded and the contents were mixed by rotating the unit gently and then the unit was left over night for reaction (it can be kept inside an incubator at 37°C for 2 h). The excess acid, left in inner chamber, was titrated against 0.02(N) NaOH using a drop of Toshiro‘s indicator. A reagent blank was done simultaneously.

Calculation: TVBN (mg %) = 14 x N x(X-Y) x 50 x 100 / W

Where, N = normality of H_2SO_4, X= ml of standard NaOH required for titration of sample, Y= ml of standard NaOH required for blank, W= weight of fish

3.4.6. Peroxide value (PV) (Jacobs, 1958)

The peroxide value of an oil or fat is the amount of peroxides (generated when lipids undergo oxidation by atmospheric oxygen through free chain

mechanism) present, expressed as milli-equivalents of peroxide oxygen per kilogram of fat. The sample is treated with potassium iodide and the iodine which is liberated by the peroxides is titrated with standard sodium thiosulphate solution.

Reagents:

(1) Solvent: 2 volumes of glacial acetic acid and 1 volume of chloroform are mixed.(2) Saturated potassium iodide solution: KI is dissolved in distilled water in 4:3 ratios and stored in brown bottle. (3) 0.01(N) sodium thiosulphate solution.
(4) Starch indicator: 0.5%

Procedure: A known volume (5-10 ml) of chloroform extract was taken in a dried and weighed petridish which was placed over a hot plate to evaporate chloroform and weight of the oil was determined from the difference of weight. 5-10 ml of chloroform extract was taken in a 250 ml iodine flask and 25 ml of solvent was added to it followed by 1 ml KI solution (or about 1 g KI salt). The content was shaken well for one minute and allowed to stand in dark for 30 min. Then about 35 ml of distilled water was added by washing the stopper and sides of the flask. The contents in the flask were titrated against 0.01N sodium thiosulphate solution using starch as indicator with vigorous shaking till first complete disappearance of blue colour. A blank was also done simultaneously with solvent only.

Calculation:

Peroxide value (milli-equivalent peroxide O2 per 1000 g fat) = 1000 (V – X) N / W
Where, V = vol. of sodium thiosulphate used for sample, X = vol. of sodium

thiosulphate used for blank, N = normality of sodium thiosulphate, W = weight of sample

3.4.7. Thiobarbituric acid (TBA)

Thiobarbituric acid (TBA) value (Tarladgis et al., 1960) is estimated to detect the lipid oxidation at later stage of oxidative rancidity. It relates the levels of aldehydes present, i.e. malonaldehyde in the sample. TBA reacts with malonaldehyde, which is produced due to methylene separated from fatty acid (dienoic or polyenoic) decomposition, to give a red chromogen, which is determined spectrophotometrically. Fat is sometimes oxidized in the presence of TBA, so more TBA reagent is added to produce colour pigment. The intensity of the colour is measured at 538 nm.

Reagents:

(1) 0.02M TBA reagent: 0.2883 g thiobarbituric acid is dissolved in 100 ml 90% glacial acetic acid by slight warming the mixture in a boiling water bath. The flask is wrapped in black foil and is prepared fresh daily. (2) HCl solution: 1 part conc. HCl to 2 parts distilled water (approx. 4N (v/v).

Procedure: Ten grams sample was macerated with 47.5 ml distilled water to a slurry form. The content was then transferred to a 500 ml round bottom flask. To this solution, 2.5 ml of HCl solution was added to adjust the pH to 1.5. The TBA distillation unit was flashed with steam and the sample flask was placed. After heating, first few drops of distillate were discarded and then distillate was collected in a measuring cylinder. Distillation was done in such a way that 50 ml of distillate was collected within 10 min. 5 ml of distillate was taken in a test tube

and 5 ml TBA reagent was mixed with it. The tubes were kept in boiling water beaker for 30 min with continuous boiling. A test tube containing 5 ml distilled water and 5 ml TBA reagent was used as blank. The tubes were cooled for 10 min and OD was measured at 538 nm.

Calculation:

TBA value (mg malonaldehyde per kg) = Absorbance X 390 / Vd X Ms

Where, A = absorbance at 538 nm, Vd = volume of distillate taken, Ms = mass of sample used

3.4.8. Microbial analysis

3.4.8.1. Sample preparation

Ten grams sample was aseptically and accurately weighed taking in a sterile aluminum petridish. This was transferred to a sterile glass mortar and macerated with 90 ml sterile diluant (0.85% NaCl solution) (10^{-1} dilution). A serial dilution was done according to the expected number of bacteria in the sample such as 10^{-2}, 10^{-3}, 10^{-4}……

3.4.8.1.2. Total plate count (TPC)

Total plate count also called as total viable count was done following spread plate technique on plate count agar following the standard method given in APHA (2001). About 15-20 ml of sterile media was poured into empty sterile petriplates, allowed to settle and dried in the laminar flow under UV exposure. Transferred 0.1ml of inoculums of each dilution to triplicate plates of nselective media (Plate Count Agar) and spread with a sterile bent glass rod. The petriplates were incubated at 37°C for 24 hours.

3.4.9. Sensory evaluation

A ten member trained and non-trained panel group evaluated the treated and untreated sardine samples at regular sampling periods during chilled storage. The panellists were instructed to evaluate all samples as per 9 point hedonic scale. They were asked to evaluate the sensory attributes, appearance, colour, odour, texture and overall acceptability of fish treated and untreated sardine.

3.4.10. Texture profile analysis

The texture of treated and control fish samples was analyzed in Universal Testing Machine (TA-PLUS Texture Analyzer, Lloyd instruments, U.K.) attached with a cylindrical plunger having 1.8 cm diameter and 5 cm height at a depression speed of 1mm per minute. The hardness1, hardness2, cohesiveness, springiness and chewiness of treated and control fish sample texture were computed and expressed in kgf, and springiness in mm.

3.4.11. Statistical analysis

Experiments and analyses were conducted in triplicate. Data obtained were appraised using Statistical Package for Social Sciences (SPSS, version 25). Analysis of variance (one way - ANOVA) was performed to determine the differences between the treatments. The tests for differences were done by using Duncan's Multiple Comparison Test. Significance of differences was defined at $P<0.05$.

IV. RESULTS

The results were documented objective-wise on the basis of antioxidant activity of pomegranate, orange, pineapple and their combined peel extracts and their effects on the storage stability of sardine. The antioxidant activity of pomegranate, orange, pineapple and combined peel was recorded. The storage stability of sardine treated with natural antioxidants extracted from pomegranate, orange, pineapple and their combined peelings was evaluated during chilled storage condition.

4.1. Total extraction yield from pomegranate orange and pineapple peels

All fruit peels were extracted u sing methanol and concentrated in rotary evaporator at low pressure and temperature. The results of the total extraction yield are presented in Table 1. The highest yield was obtained by the dried pomegranate peel powder, 23.74% and orange peel powder, 20.06% where as in case of pineapple peel powder the total extract obtained was 17.11%.

4.2. Total phenolic content (TPC) and total flavonoid content (TFC)

TPC of the pomegranate peel extract (PPE), orange peel extract (OPE), pineapple peel extract (PAPE) and combined fruit peel extracts (CFPE) were estimated by the folin-ciocalteu methods and results were expressed in terms of Gallic acid/equivalents. The Gallic acid standard curve was prepared in the concentration range of 20 µg/mL to 100 µg/mL and values were plotted as concentration v/s absorbance. The results of total phenolic compounds of PPE, OPE, PAPE and CFPE are showed in Table 2. The TPC values of the PPE was higher (226 mg/g GAE) than CFPE (152 mg/g GAE), OPE (141.66 mg/g GAE)

and PAPE (137 mg/g GAE). The total flavonoid content was measured by aluminum chloride method and the results expressed in terms of quercetin equivalent. The standard curve of quercetin was prepared at concentration of 20 µg/mL to 100 µg/mL. The results of total flavonoid compounds are presented in the Table3. A higher value of 189.33 mg/g QE in PAPE as compared to PPE i.e.177.33 mg/g QE, CFPE 111.33 mg/g QE and OPE 88.66 mg/g QE respectively.

4.3. DPPH radical scavenging activities of the different fruit peel and their combined peel extracts

The DPPH radical scavenging activity of PPE, OPE, PAPE and CFPE are shown in Table 4 and Figure1. The DPPH radical scavenging activity of different fruit peel and their combined peel extracts showed a different concentration range i.e. 2 µg/mL to 10 µg/mL. PPE extracts showed radical scavenging activity only at the concentration of 4 µg/mL with the inhibitory activity of 88.46% at the same concentration BHA was almost equivalent to 91.87% while CFPE, PAPE and BHT showed the radical scavenging activity at a concentration of 6 µg/mL i.e. 83.09%, 76.85%, 89.54% respectively and OPE showed the radical scavenging activity at a concentration of 8 µg/mL i.e. 81.53 respectively.

4.4. Proximate Composition of Fresh Sardine (*Sardinella gibossa)*

The proximate composition of fresh sardine is presented in Table 5. The moisture, crude protein, total lipid and ash contents were determined (% wet weight basis) as 78.78, 19.18, 2.66, 1.45 respectively.

Table 1. Total extraction yield from pomegranate, orange and pineapple peels

Extracts	Yield (%)
PPE	23.74
OPE	20.06
PAPE	17.11

(Note: PPE- pomegranate peel extract, OPE – orange peel extract, PAPE – pineapple peel extract)

Table 2. Total phenolic content (mg/g GAE) of fruit peel and their combined peel extracts

Extracts	Total phenolic content
PPE	226±31.72
OPE	141.66±34.02
PAPE	137.33±13.47
CFPE	152±20.83

(Note: PPE- pomegranate peel extract, OPE – orange peel extract, PAPE – pineapple peel extract, CFPE- combined fruit peel extracts)

Table 3. Total flavonoid content (mg/g QE) of fruit peel and their combined peel extracts

Extracts	Total flavonoid content
PPE	177.33±22.89
OPE	88.66±13.47
PAPE	189.33±10.20
CFPE	111.33±33.10

(Note: PPE- pomegranate peel extract, OPE – orange peel extract, PAPE – pineapple peel extract, CFPE- combined fruit peel extracts)

Table 4. DPPH scavenging activity (%) of different fruit peel and their combined peel extracts, BHA and BHT

Concentration (µg/ml)	Extracts (DPPH %)					
	PPE	OPE	PAPE	CFPE	BHA	BHT
2	66.12±33.47	57.76±26.72	58.41±25.54	62.86±28.50	67.94±30.96	65.32±28.88
4	88.46±38.19	75.16±32.92	68.24±29.91	70.27±30.63	91.87±39.40	73.12±31.93
6	75.66±32.35	69.65±30.35	76.85±32.53	83.09±35.39	78.92±33.39	89.54±37.5
8	70.01±29.60	81.53±33.28	63.16±29.19	74.93±31.17	73.42±30.06	77.00±31.37
10	77.88±31.49	74.26±30.28	70.17±27.22	74.70±29.31	80.89±32.24	81.69±32.81

(Note: PPE- pomegranate peel extract, OPE – orange peel extract, PAPE – pineapple peel extract, CFPE- combined fruit peel extracts)

Table 5. Proximate composition of Sardine

Parameters	Results
Moisture (%)	78.78±0.68
Protein (%)	19.18±0.43
Lipid (%)	2.66±0.18
Ash (%)	1.45±0.09

4.5. Standardization of application of different fruit peel and their combined peel extracts on sardine under chilled storage condition at 4±1˚C

4.5.1 Biochemical changes during chilled storage at 4±1°C of different fruit peel and their combined peel extracts, BHA and BHT treated Sardine

Biochemical changes of sardine treated with fruits peel extracts was studied during storage at 4±1°C for 72 hours. The data is presented in Tables 6 to 8 and in Figures 2 to 4.

4.5.1.1. Changes in peroxide value of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

It was found that the peroxide value (milli-equivalent peroxide O_2 /kg lipid) increased with the increase of chilled storage. In control, the peroxide value was found to increase from 2 to 3.56 at the end of 3 days of storage period. In case of TP1, the PV was found to increase from 1.46 - 2.53 meq/kg and TP3, the PV was found to increase from 1.16 - 2.16 meq/kg. In case of OP1 and OP3 Peroxide values were1.9 - 2.6 and 1.4 – 2.4 meq/kg respectively in respect of corresponding storage period. In case of TPA1 and TPA3 peroxide value was

1.83 – 2.86 and 1.5 - 2.03 meq/kg respectively. TCF1 and TCF3 initial peroxide value found was 1.6 and 1.33 final value was 2.53 and 2.33 meq/kg. In case of TB and TT the peroxide value was found to increase from 1.2 – 2.2 and 1.3 - 2.4 meq/kg at the end of 3rd day of storage period.

4.5.1.2. Changes in thiobarbituric acid of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

During the period of storage, it was found that the TBA values (mg malonadehyde /kg) increased in all the treatments. In control, the TBA increased from 0.79 to 1.61 mg MDA/kg. In TP1, the TBA was found to increase from 0.69 to 0.78 mg MDA/kg at the end of the storage days. In TP3, the TBA was found to increase from 0.56-0.64 mg MA/kg. In case of TO1 and TO3, TBA value were 0.70 – 0.80 and 0.64 – 0.75 respectively in respect of corresponding storage period. In case of TPA1 and TPA3, TBA values were 0.71-0.81 and 0.69 – 0.77 mg MDA/kg respectively in respect of corresponding storage period. TCF1 and TCF3 initial TBA value were found that 0.70 and 0.68 final value was found 0.79 and 0.74 mg MDA/kg. TB and TT initial TBA value were found 0.58-0.67 and 0.6-0.71 mg MDA/kg respectively.

4.5.1.3. Changes in total volatile basic nitrogen of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

The quality index, TVBN (mg %) increased with the progress of storage period. In control, the TVBN (mg %) was found to increase from 14.93 to 16.08 mg/100 g at the end of storage period. In TP1, the TVBN value was found to

increase from 13.06 -14.93 mg %. In TP3 the TVBN value was found to increase from 12.13 -14 at the end of storage period days. In case of TO1 and TO3, TVBN value was 13.06-15.86 and 13.06-14.93 mg % respectively in respect of corresponding storage period. In case of TPA1 and TPA3, TVBN values were 14 – 15.86 and 13.06-14.93 mg % respectively. In TCF1 and TCF3 initial TVBN value were found that 13.06 and 13.06 final value were found 15.86 and 14.93 mg/100 g. In case of TB and TT initial TVBN value were found 12.13 - 14 and 12.13 – 14 mg % respectively.

4.5.2. Microbial changes during chilled storage of fruit peel and their combined peel extracts, BHA and BHT treated Sardine at 4±1°C

4.5.2.1. Changes in total plate count (TPC) of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

During the storage period the TPC (total plate count in log cfu g-1) increased along the storage period. In control the TPC was found to increase from 3.73 to 3.93 log cfu g-1 at the end of storage of 3 days. In case of TP1 and TP3, the TPC was found to increase from 3.4-3.8 and 3.2-3.5 respectively. In TO1 and TO3, TPC was found to increase from 3.36-3.83 and 3.43-3.66 at the end of the storage of days. In case of TP1 and TP3, the TPC were 3.6-3.86 and 3.46-3.7 respectively in respect of corresponding storage period. In TCF1 and TCF3 initial TPC were found that 3.5 and 3.36 final value was found 3.8 and 3.6 respectively. Similarly TB and TT treated with sardine fish of the initial TPC was found 3.06-3.43 and 3.1-3.5 respectively. The data are presented in Table 9 and Fig.5.

Table 6. Changes in peroxide value (meq O_2/kg) of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	1.46±0.04	2.16±0.12	2.53±0.04
TP3	1.16±0.04	1.8±0.08	2.16±0.12
TO1	1.9±0.08	2.03±0.04	2.6±0.08
TO3	1.4±0.08	1.9±0.08	2.43±0.04
TPA1	1.83±0.04	2.56±0.04	2.86±0.04
TPA3	1.5±0.081	2.03±0.04	2.03±0.04
TCF1	1.6±0.08	2.23±0.04	2.53±0.04
TCF3	1.33±0.04	2.06±0.04	2.33±0.04
TB	1.2±0.08	1.9±0.08	2.2±0.08
TT	1.3±0.08	2±0.08	2.4±0.08
CON	2±0.16	2.83±0.04	3.56±0.09

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 7. Changes in thiobarbituric acid (mg MDA/kg) of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	0.69±0.00	0.72±0.00	0.78±0.00
TP3	0.56±0.00	0.59±0.00	0.64±0.00
TO1	0.70±0.00	0.75±0.00	0.80±0.00
TO3	0.64±0.00	0.69±0.00	0.75±0.00
TPA1	0.71±0.00	0.76±0.00	0.81±0.00
TPA3	0.69±0.00	0.73±0.00	0.77±0.00
TCF1	0.70±0.00	0.76±0.00	0.79±0.00
TCF3	0.68±0.00	0.71±0.00	0.74±0.00
TB	0.58±0.00	0.61±0.00	0.67±0.00
TT	0.6±0.00	0.65±0.00	0.71±0.00
CON	0.79±0.00	0.87±0.00	1.61±0.33

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 8. Changes in total volatile base nitrogen (mg/100g) of sardine treated with different fruit peel and their combined peel extracts, BHA & BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	13.06±2.63	14±2.28	14.93±1.31
TP3	12.13±5.75	13.06±1.31	14±9.96
TO1	13.06±3.49	14.93±5.27	15.86±1.31
TO3	13.06±8.65	14±9.96	14.93±11.27
TPA1	14±9.96	14.93±5.75	15.86±1.31
TPA3	13.06±1.31	14±2.28	14.93±5.27
TCF1	13.06±5.75	14.93±11.27	15.86±5.75
TCF3	13.06±3.49	14±9.96	14.93±5.27
TB	12.13±3.49	13.06±1.31	14±4.57
TT	12.13±5.75	13.06±8.65	14±9.96
CON	14.93±6.59	15.86±1.31	16.8±4.57

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 9. Changes in total plate count (cfu/g) of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	3.4±1.45	3.63±1.51	3.8±1.76
TP3	3.2±1.57	3.33±1.32	3.56±1.35
TO1	3.63±1.66	3.76±0.86	3.83±0.97
TO3	3.43±1.09	3.5±1.46	3.66±1.81
TPA1	3.6±1.23	3.73±0.75	3.86±0.74
TPA3	3.46±0.83	3.53±1.83	3.7±1.76
TCF1	3.5±1.27	3.7±1.12	3.8±0.77
TCF3	3.36±1.11	3.46±0.96	3.6±1.26
TB	3.06±1.39	3.33±1.26	3.43±1.3
TT	3.1±1.41	3.33±1.3	3.5±1.34
CON	3.73±1.32	3.8±0.77	3.93±0.7

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

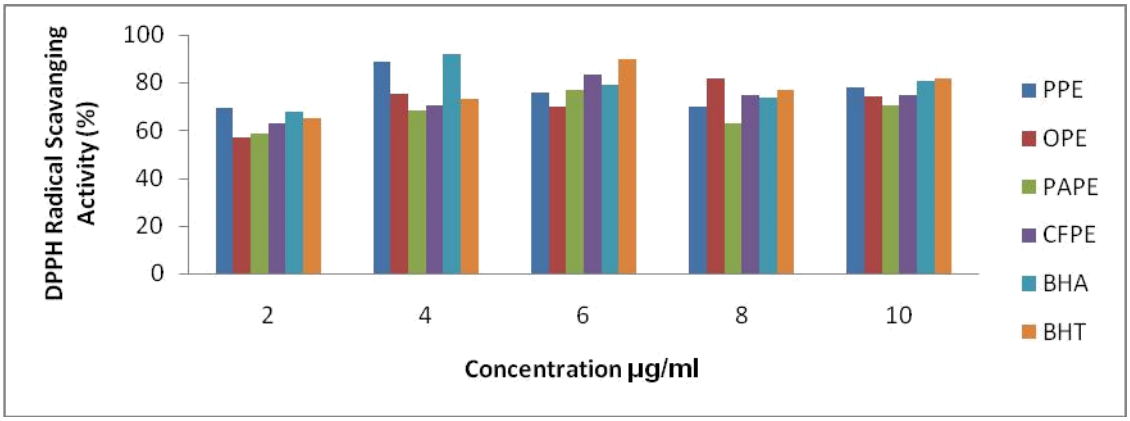


Fig.1. DPPH scavenging activity (%) of fruit peel extracts, BHA and BHT

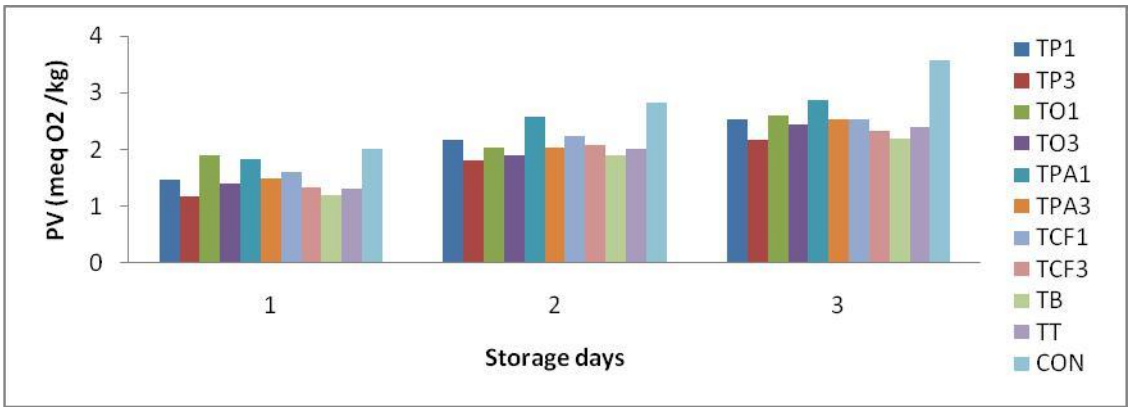


Fig. 2. Changes in peroxide value of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

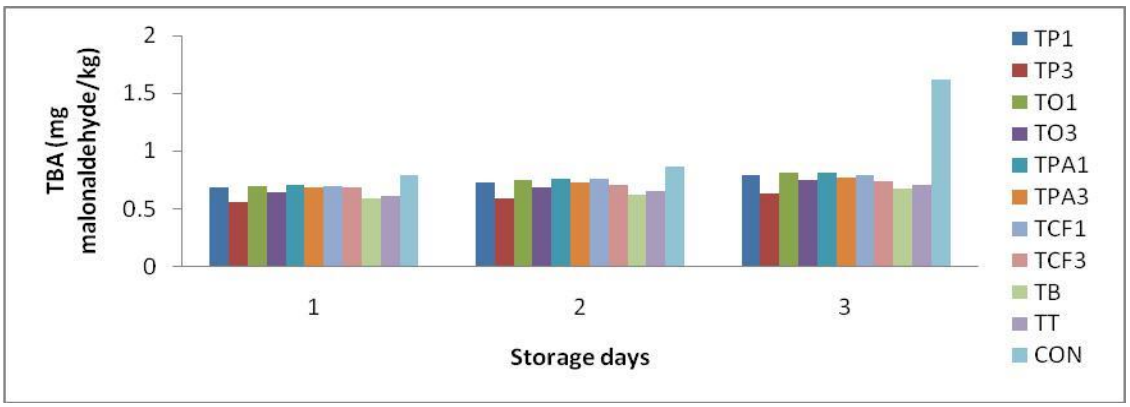


Fig. 3. Changes in thiobarbituric acid of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

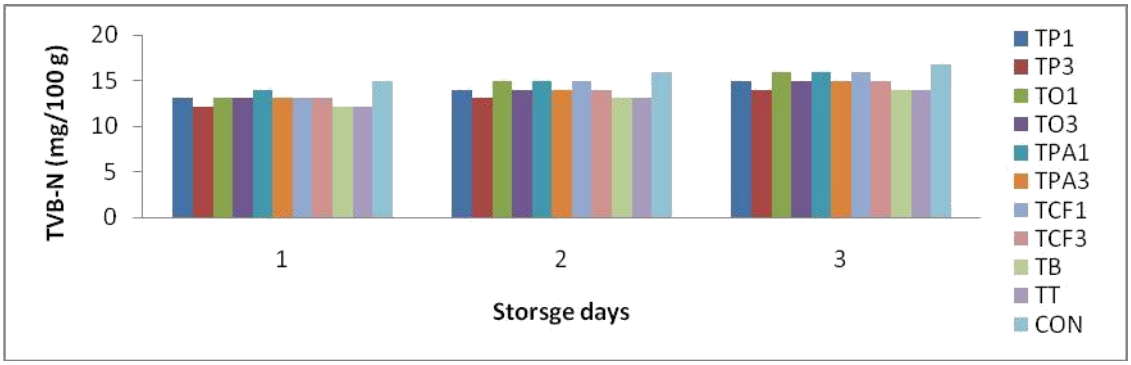


Fig. 4. Changes in total volatile base nitrogen of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

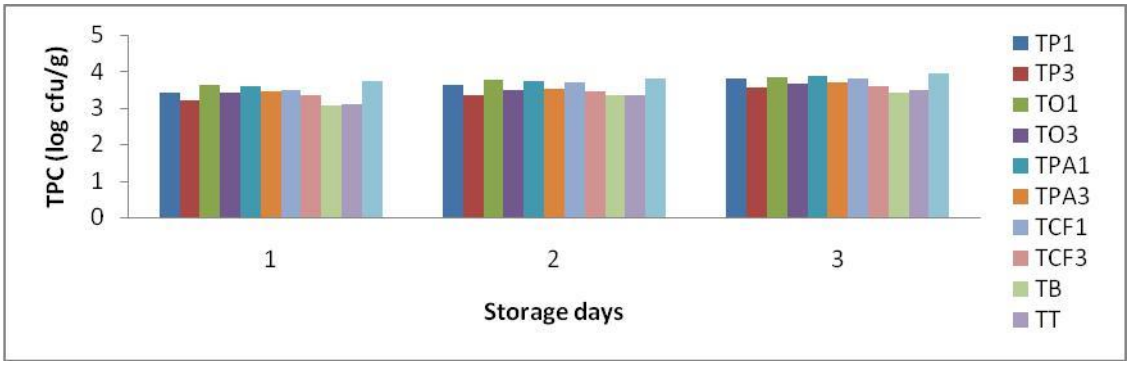


Fig. 5. Changes in total plate count (TPC) of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

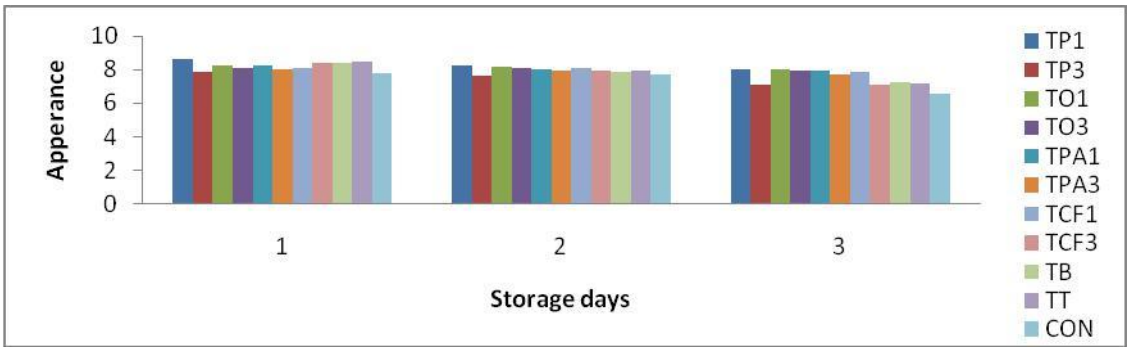


Fig. 6. Changes in appearance of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

4.5.3. Sensory changes during chilled storage at 4±1°C of different fruit peel and their combined peel extracts, BHA and BHT treated Sardine

Treated and control sardine was assessed for sensory evaluation for three days in order to assess the shelf life as well as the difference among the treatment sample and control are presented in Tables 10 to 14 and Fig. 6 to 10. All the sensory attributes decreased but to an acceptable range during the storage period.

The overall acceptability scores during chilled storage period are presented in Table 14 and Fig.10. In control, the initial value of overall acceptability was 7.85 and the final value was 6.69 observed. The value was decreased with storage time. In case of TP1, TP3, TO1, TO3, TPA1 TPA3, TCF1, TT and TB, the value was significantly decreased (<0.05) with storage time. The initial value was 8.66, 8.01, 8.21, 8.08, 8.25, 8.48, 8.46, 8.55, 8.29, and 8.24 observed and the final value was7.78, 7.39, 7.86, 7.65, 7.87, 7.73, 7.87, 7.43, 7.34, and 7.17 respectively. In the results showed among all treatments the TP1 and TCF1 was more acceptable followed by TO1<TP1<TPA3<TO3<TCF<TP3<TB< TT.

Table 10. Changes in sensory attribute - appearance of sardine treated with different fruit peel and their combined peel extracts, BHA & BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	8.65±0.55	8.23±0.78	8.05±0.91
TP3	7.84±0.47	7.62±0.47	7.12±0.60
TO1	8.17±0.36	8.24±0.62	7.99±0.74
TO3	8.07±0.51	8.12±0.74	7.94±.66
TPA1	8.22±0.45	7.99±1.13	7.92±0.53
TPA3	8.01±1.06	7.94±0.74	7.7±0.36
TCF1	8.12±0.58	8.08±1.02	7.9±0.34
TCF3	8.42±0.88	7.97±1.29	7.08±0.61
TB	8.43±0.66	7.84±0.90	7.22±0.84
TT	8.49±0.66	7.97±1.06	7.17±1.00
CON	7.79±1.14	7.74±1.00	6.56±1.00

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 11. Changes in sensory attribute - colour of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	8.45±0.56	7.75±0.52	7.41±0.75
TP3	8±0.31	7.47±0.46	7.09±0.63
TO1	8.18±0.34	8.07±0.64	7.9±0.34
TO3	8.02±0.81	7.97±0.41	7.81±0.67
TPA1	8.14±1.13	7.91±0.52	7.81±0.74
TPA3	8.33±0.72	8.1±0.74	7.65±0.45
TCF1	8.13±1.12	7.95±0.96	7.92±0.47
TCF3	8.35±0.64	8.15±0.96	7.23±0.99
TB	8.18±0.56	7.74±0.81	7.42±0.99
TT	8.38±0.73	7.89±0.93	7.23±0.99
CON	7.89±0.91	7.82±0.94	6.63±0.90

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON – control

Table 12. Changes in sensory attribute - odour of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	8.62±0.61	7.79±0.42	7.83±.0.72
TP3	8.32±0.67	7.33±0.79	7.32±0.66
TO1	8.17±0.57	8.04±0.70	7.93±0.35
TO3	8.28±0.62	7.78±0.70	7.77±0.74
TPA1	8.28±0.96	7.8±0.37	7.34±1.14
TPA3	8.23±0.94	7.7±0.94	7.43±0.44
TCF1	8.34±0.99	7.72±1.40	7.67±0.38
TCF3	8.47±0.63	7.62±1.36	7.26±0.98
TB	8.34±0.64	7.66±0.68	7.27±0.98
TT	8.16±0.63	8.06±0.90	6.92±0.78
CON	8±0.59	7.22±1.27	6.48±0.97

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 13. Changes in sensory attribute - texture of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	8.5±0.5	8.03±0.81	7.81±0.74
TP3	8.2±0.4	7.65±0.39	7.42±0.81
TO1	8.43±0.44	8.01±0.64	7.92±0.27
TO3	8.28±0.62	7.66±0.50	7.48±0.45
TPA1	8.54±0.78	7.97±0.71	7.93±0.59
TPA3	8.37±0.56	8.21±0.77	7.68±0.42
TCF1	8.55±0.78	7.95±052	7.72±1.04
TCF3	8.78±0.32	7.95±0.92	7.43±0.90
TB	8.39±0.67	7.68±0.71	7.13±0.85
TT	8.31±0.79	8.22±0.86	7.3±1.14
CON	8.02±0.67	7.94±0.56	6.84±0.97

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 14. Changes in sensory attribute - overall acceptability of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	8.66±0.52	7.96±0.73	7.78±0.69
TP3	8.01±0.30	7.4±0.45	7.39±0.52
TO1	8.21±0.344	8.12±0.69	7.86±0.43
TO3	8.08±0.51	7.79±0.69	7.65±0.48
TPA1	8.25±0.98	8.03±0.61	7.87±0.96
TPA3	8.48±0.61	8.34±0.81	7.73±0.42
TCF1	8.46±1.00	8.15±1.20	7.87±0.42
TCF3	8.55±0.62	7.77±1.16	7.43±0.49
TB	8.29±0.60	7.54±0.51	7.34±0.99
TT	8.24±0.72	8.2±0.72	7.17±0.96
CON	7.85±0.86	7.7±0.75	6.69±0.81

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 15. Changes in texture profile - harness1 of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	1.01±0.00	1.01±0.00	1.01±.00
TP3	1.11±0.16	0.98±0.09	0.96±0.11
TO1	0.87±0.18	0.78±0.00	0.73±0.04
TO3	0.94±0.10	0.65±0.05	0.62±0.13
TPA1	0.75±0.00	0.93±0.04	0.90±0.01
TPA3	1.00±0.16	0.77±0.06	0.76±0.00
TCF1	0.86±0.05	0.82±0.04	0.82±0.01
TCF3	0.78±0.08	0.59±0.05	0.56±0.05
TB	0.87±0.17	0.87±0.05	0.87±0.05
TT	0.67±0.00	0.73±0.05	0.71±0.14
CON	0.72±0.04	0.77±0.06	0.77±0.14

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

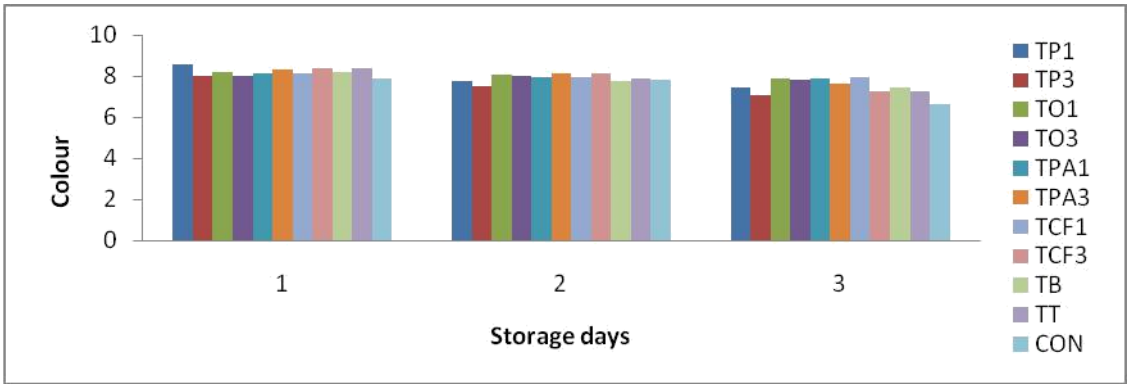


Fig.7. Changes in colour of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

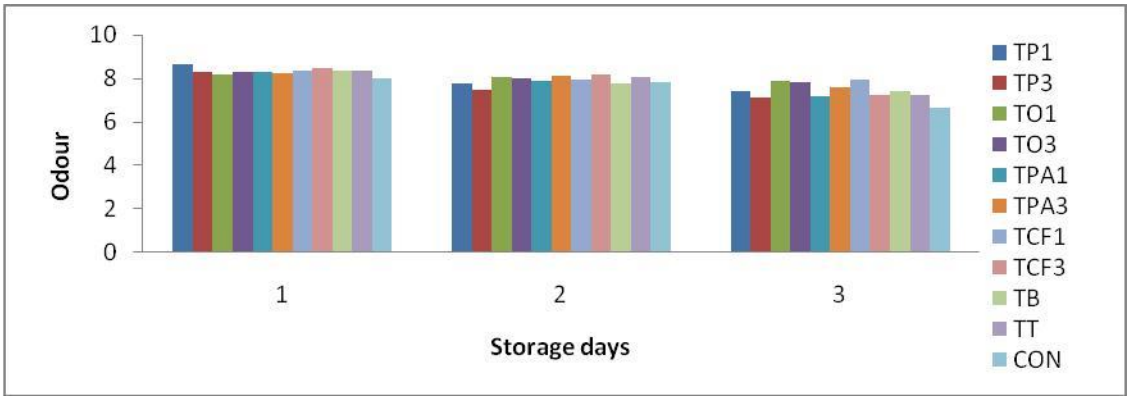


Fig.8. Changes in odour of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

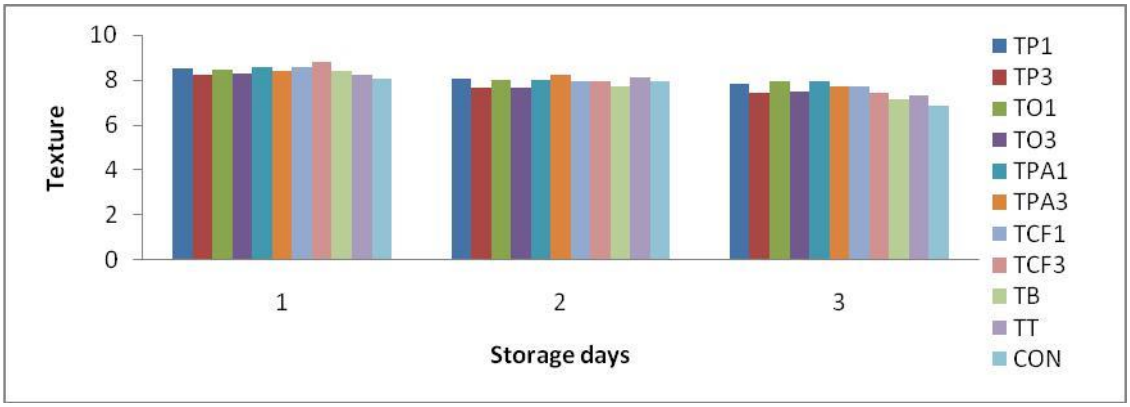


Fig.9. Changes in texture of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

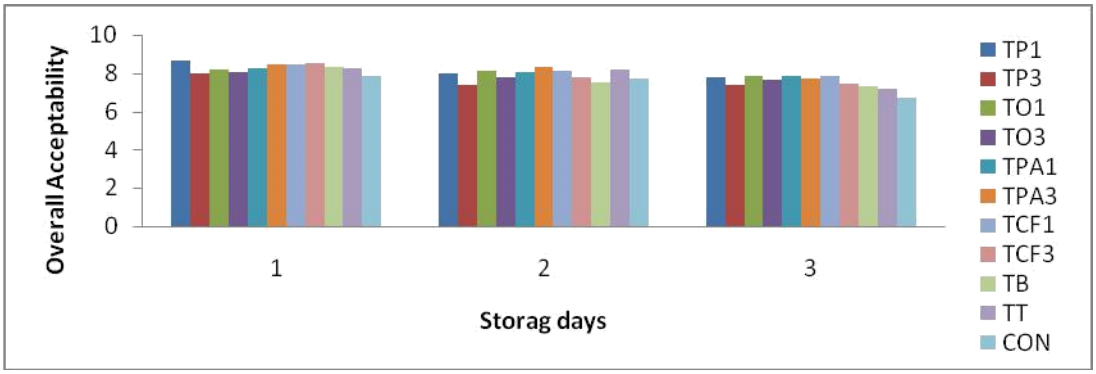


Fig.10. Changes in overall acceptability of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

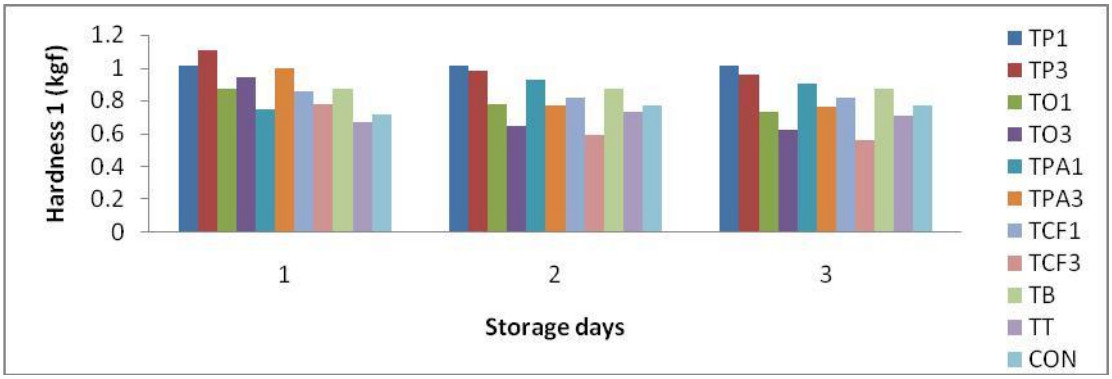


Fig.11. Changes in harness1 of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

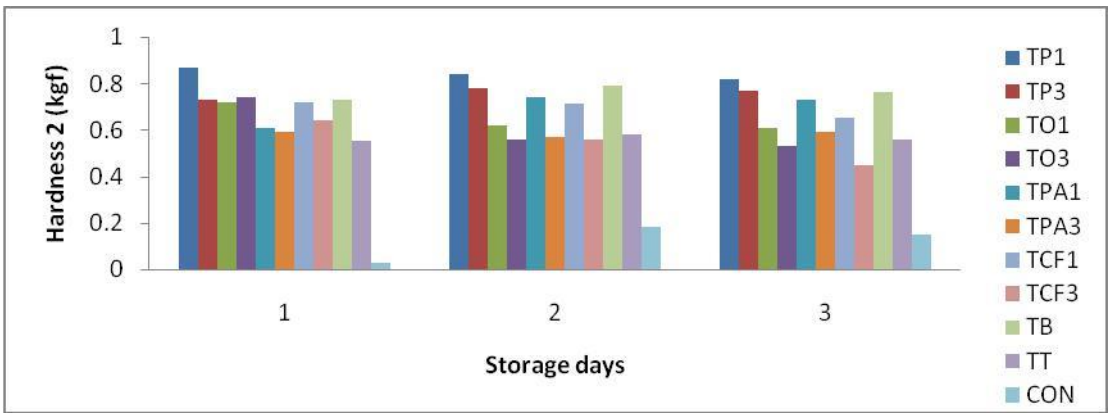


Fig.12. Changes in harness2 of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

4.5.4. Texture profile analysis (TPA) chilled storage at 4±1°C of different fruit peel and their combined peel extracts, BHA and BHT treated Sardine

During three days of chilled storage, some indices of treated and control fish showed decreasing trends (i.e. hardness1, Hardness 2, springiness, cohesiveness, springiness and chewiness). The data are represented in Table 15 to 19 and Fig.11 to15.

4.5.4.1. Changes in hardness 1 of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

During chilled storage in the control, the hardness 1 initial value was 0.72 and was constant till the end of 3 days of storage. In case of TPA1 and TT, the hardness 1 initial value was 0.75 and 0.67 it increased up to two day after that it decreased respectively. In case of TP1, TP3, TO1, TO3, TPA3, TCF1, TCF3 and TB samples, the hardness 1 initial value was 1.01, 1.11, 0.87, 0.94, 1.00, 0.86, 0.78, and 0.67 kgf respectively and then it decreased with increased in storage time.

4.5.4.2. Changes in hardness 2 of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

During chilled storage the TP1, TO1, TO3, TPA1,TPA3, TCF1 and TCF3 samples, the hardness 2 initial value was 0.87, 0.63, 0.72, 0.74, 0.61, 0.72, and 0.64 kgf respectively after that the value decreased. In case of TPA3, the initial value of hardness 2 was found to be 0.59 kgf and after that it decreased and

again increased at the end of 3 days of storage period. Where as in case of TP3, TB, TT, and control from the initial value 0.63, 0.73, 0.55 and 0.03 kgf respectively it progressively increased and then decreased.

4.5.4.3. Changes in cohesiveness of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

During chilled storage the initial value of TP1, TO1, TPA1, TPA3 and TCF1 samples, was 0.33, 0.30, 0.32, 0.32 and 0.28 respectively, it decreased with storage time. In case of TPA3, TO3, TCF3, TB, TT and control, the initial value of cohesiveness was increased to 0.31, 0.31, 0.28, 0.27, 0.27, 0.29 and 0.27 respectively. After that the values were found to be decreasing at the end of 3 days of storage period.

4.5.4.4. Changes in springiness of sardine treated with different peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

The initial value of springiness TP1, TP3 and TPA1 samples, the springiness initial value was found 1.66, 1.66 and 1.57 mm respectively, the value was increased with storage time. In case of TO1, the initial value of springiness was 1.47 it was constant till the end of storage time. In case of TCF1, TCF3, TB, TT and control sample, the springiness initial i.e. 1.55, 1.48, 1.49, 1.56 and 1.46 mm respectively increased and it was decreased with storage time.

4.5.4.5. Changes in chewiness of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

For the samples TP1, TP3, TO1, TO3, TPA3 and TCF3, the chewiness initial value was 0.59, 0.62, 0.40, 0.44, 0.58 and 0.36 kgf respectively, the value decreased with storage time. Whereas, in case of TPA1, TCF1, TB, TT and control, initially the cohesiveness was 0.36, 0.25, 0.41, 0.34 and 0.28 kgf respectively. It increased and then was decreased at the end of 3rd day of storage.

Table 16. Changes in texture profile - harness 2 of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	0.87±0.02	0.84±0.05	0.82±0.00
TP3	0.63±0.40	0.78±0.05	0.77±0.08
TO1	0.72±0.14	0.62±0.10	0.61±0.03
TO3	0.74±0.07	0.56±0.07	0.53±0.11
TPA1	0.61±0.00	0.74±0.04	0.73±0.01
TPA3	0.59±0.39	0.57±0.03	0.59±0.02
TCF1	0.72±0.08	0.71±0.12	0.65±0.02
TCF3	0.64±0.05	0.56±0.16	0.45±0.04
TB	0.73±0.14	0.79±0.07	0.76±0.02
TT	0.55±0.01	0.58±0.05	0.56±0.08
CON	0.03±0.28	0.18±0.26	0.15±0.28

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 17. Changes in texture profile - cohesiveness of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatment	1st day	2nd day	3rd day
TP1	0.33±0.03	0.31±0.03	0.29±0.00
TP3	0.31±0.00	0.32±0.04	0.31±0.02
TO1	0.30±0.00	0.28±0.02	0.27±0.03
TO3	0.31±0.01	0.34±0.04	0.32±0.00
TPA1	0.32±0.02	0.31±0.02	0.30±0.00
TPA3	0.32±0.02	0.32±0.02	0.31±0.01
TCF1	0.28±0.01	0.33±0.04	0.31±0.00
TCF3	0.27±0.01	0.30±0.00	0.29±0.00
TB	0.27±0.00	0.34±0.04	0.33±0.01
TT	0.29±0.01	0.33±0.05	0.30±0.00
CON	0.27±0.01	0.29±0.07	0.27±0.01

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 18. Changes in texture profile - springiness of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatment	1st day	2nd day	3rd day
TP1	1.66±0.08	1.57±0.03	1.66±0.01
TP3	1.66±0.01	1.30±0.22	1.51±0.12
TO1	1.47±0.05	1.53±0.08	1.53±0.05
TO3	1.62±0.07	1.49±0.08	1.47±0.08
TPA1	1.57±0.01	1.52±0.05	1.57±0.03
TPA3	1.66±0.05	1.55±0.02	1.53±0.02
TCF1	1.55±0.06	1.67±0.16	1.63±0.00
TCF3	1.48±0.08	1.47±0.05	1.43±0.01
TB	1.49±0.07	1.72±0.13	1.71±0.03
TT	1.56±0.07	1.66±0.15	1.64±0.02
CON	1.46±0.03	1.54±0.04	1.50±0.12

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

Table 19. Changes in texture profile - chewiness of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1°C

Treatments	1st day	2nd day	3rd day
TP1	0.59±0.10	0.53±0.04	0.50±0.00
TP3	0.62±0.11	0.53±0.04	0.44±0.12
TO1	0.40±0.08	0.34±0.03	0.33±0.01
TO3	0.44±0.05	0.32±0.03	0.29±0.08
TPA1	0.36±0.00	0.47±0.09	0.45±0.01
TPA3	0.58±0.14	0.49±0.14	0.38±0.01
TCF1	0.25±0.15	0.46±0.07	0.44±0.00
TCF3	0.36±0.03	0.30±0.06	0.25±0.01
TB	0.41±0.11	0.55±0.10	0.51±0.02
TT	0.34±0.04	0.48±0.08	0.40±0.03
CON	0.28±0.02	0.35±0.10	0.30±0.11

Note: TP1 & TP3 – pomegranate 1% & 3%, TO1 & TO3 – Orange 1% & 3%, TPA1 & TPA3 – pineapple 1% & 3%, TB – BHA, TT- BHT, CON - control

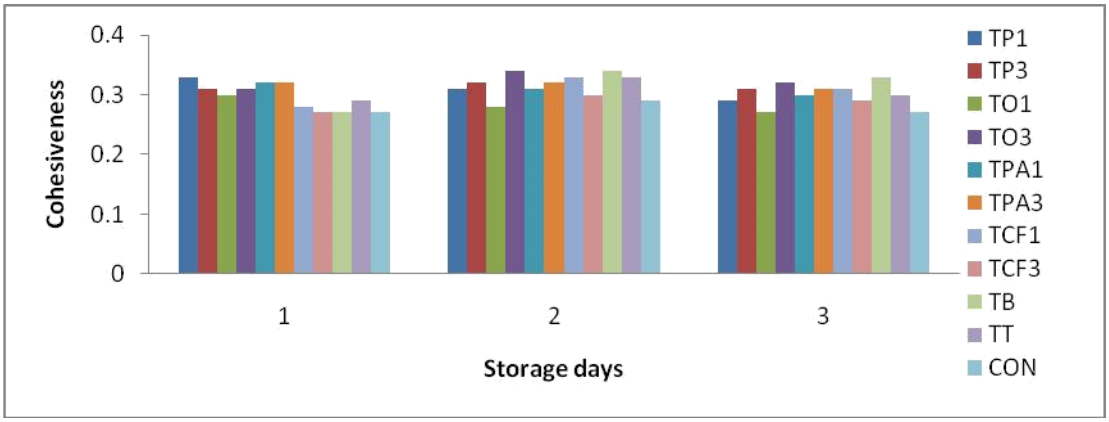


Fig.13. Changes in cohesiveness of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1˚C

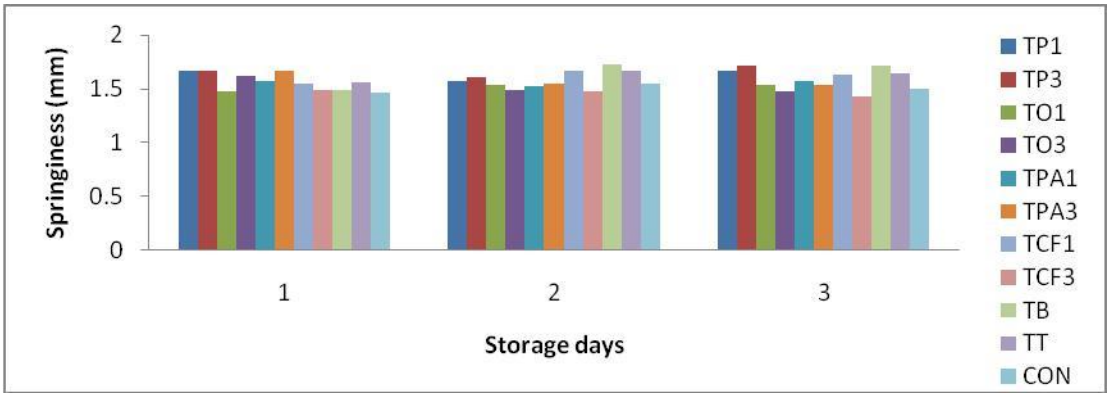


Fig. 14. Changes in springiness of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1˚C

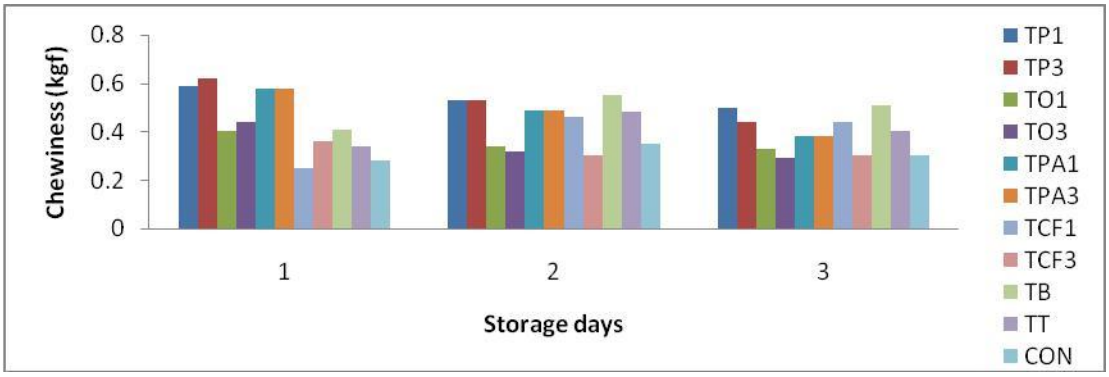


Fig.15. Changes in chewiness of sardine treated with different fruit peel and their combined peel extracts, BHA and BHT during chilled storage condition at 4±1˚C

4.6. Storage study of pomegranate peel extract and BHA treated sardine under chilled storage condition at 4±1˚C

After standardization the results showed that among the different and combined fruit peel extracts, pomegranate peel extracts treated fish and BHA treated fish gave better storage stability. So pomegranate peel extract was selected and BHA treated sardine fish for 15 days chilled storage study.

4.6.1. Biochemical changes during chilled storage at 4±1°C of pomegranate peel extract and BHA treated Sardine

Biochemical changes of sardine treated with fruits peel extracts was studied during storage at 4±1°C for 15 days of chilled storage. The data are presented in Tables 20 to 22 and in Figures 16 to 19.

4.6.1.1. Changes in peroxide value of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

The changes in peroxide value of TP1, TP3 and TB during chilled storage are presented in the Table 20 and Fig.16. Peroxide value of control significantly increased ($p<0.05$) up to 9^{th} day whereas from 12^{th} day onwards the value is decreased till storage periods. The 0^{th} day value was 1.56 me /kg and 15^{th} day value was 13.7 meq/kg respectively. For treated fish also, the PV value significantly increased up to 12^{th} day and there after decreased till 15^{th} day of storage. The initial PV value of TP1, TP3 and TB treated fish was 1.23, 1.06, 1.13 and the final PV value was 11.53, 10.33, and 11.36 meq/kg respectively. The above results of

peroxide value indicate that the TP3 was more effective in inhibiting primary oxidation products than TP1 and TB.

4.6.1.2. Changes in thiobarbituric acid of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

The results of changes in TBA are presented in Table 21 and illustrated in the Fig. 17. The secondary oxidation products TBA gradually increased with increase in storage time. The lowest TBARS value was observed at 0^{th} day with values 0.48, 0.43, 0.46 and 0.51 mg MDA/kg of sample in TP1, TP3, TB and control respectively and there after it significantly ($p<0.05$) increased with increase in storage period. The highest TBA value was observed in case of control at end of day recorded with 6.37 and the lowest was measured in the case of sample treated with TP1, TP3 and TB (3.92, 3.55 and 3.83 mg MDA/kg). The results showed that theTP3 was most promising antioxidant in inhibiting the secondary oxidation products.

4.6.1.3. Changes in total volatile base nitrogen of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

The changes in TVB-N value in treated sardine at chilled storage are tabulated in the Table 22 and Fig.18. The results of TVB-N of all the treated samples significantly increased with increase in storage period and the rate of increase was more in case of control sample. The initial TVB-N values were 12.13 for TP1, TP3 12.13 and TB 12.13 mg %. In the control sample, TVB-N recorded a higher value in comparison with other samples and was found to be significant with respect to treated sardine samples. After 12^{th} day of storage the control sample showed the

TVB-N value of 45.73 mg % where in the sample TP1, TP3 and TB the value was 37.33, 35.46 and 36.4 mg %, respectively. The above results showed that the TP3 were more effective in controlling the formation of total volatile base- nitrogen.

4.6.2. Microbial changes during chilled storage at 4±1°C of pomegranate peel extract and BHA treated Sardine fish

Microbial changes such as total plate count (TPC) of sardine treated with pomegranate peel extract ,BHA and control was studied at 4±1°C for 15 days in chilled storage and the pomegranate peel extracts and BHA treated sardine fish data are presented in Tables 23 and Fig.19.

4.6.2.1. Changes in total plate count (TPC) of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

In the beginning of storage the TPC value observed was 2.63, 2.13, 2.5 and 2.8 log cfu/g in TP1, TP3, TB and control sample and there after it was significantly increased ($p<0.05$) with increase in storage time and this trend was more in case of control sample followed by TP1, TP3 and TB treated sardine.

Table 20. Changes in peroxide value of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Parameter	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Peroxide Value (meq O_2/kg)	TP1	1.23±0.20	2.86±016	5.64±0.19	10.64±0.19	13.23±0.12	11.53±0.12
	TP3	1.06±0.20	2±0.16	4.43±0.04	9.43±0.04	11.43±0.04	10.33±0.33
	TB	1.13±0.20	2.56±0.16	5.43±0.20	10.6±0.16	12.6±0.16	11.36±0.32
	CON	1.56±0.09	3.6±0.28	8.7±0.16	15.23±0.12	14.56±0.46	13.7±0.35

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON – control

Table 21. Changes in thiobarbituric acid of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Parameter	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Thiobarbituric Acid (mg malonaldehyde/kg)	TP1	0.48±0.07	0.83±0.02	1.73±0.11	2.65±0.11	3.34±0.01	3.92±0.02
	TP3	0.43±0.07	0.67±0.04	1.12±0.08	2.11±0.04	2.93±0.03	3.55±0.08
	TB	0.46±0.06	0.74±0.02	1.43±0.04	2.34±0.06	3.08±0.03	3.83±0.02
	CON	0.51±0.04	1.70±0.08	3.12±0.10	4.63±0.08	5.59±0.06	6.37±0.40

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

Table 22. Changes in total volatile base nitrogen of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Parameter	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Total Volatile Base Nitrogen (mg/100 g)	TP1	12.13±7.34	14±2.28	17.73±1.31	23.33±1.31	28±2.28	37.33±1.31
	TP3	12.13±5.75	13.06±8.65	16.8±4.57	22.4±2.28	26.13±1.31	35.46±1.31
	TB	12.13±3.49	14±4.57	16.8±6.68	21.46±3.49	27.06±1.31	36.4±3.95
	CON	12.13±1.31	14.93±5.27	25.2±2.28	35.46±1.31	41.06±1.31	45.73±2.63

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

Table 23. Changes in total plate count (TPC) of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Parameter	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Total Plate Count (log cfu/g)	TP1	2.63±1.30	3.63±1.77	4.23±1.74	5.26±3.25	6.03±3.06	6.93±3.71
	TP3	2.13±1	3.43±0.95	4.06±1.12	4.63±1.82	5.53±3.92	6.5±3.42
	TB	2.5±0.42	3.53±1.42	4.23±2.04	4.96±3.71	5.76±4.75	6.86±3.46
	CON	2.8±0.21	3.83±1.07	4.9±3.68	6.1±3.96	7.03±4.21	7.26±3.62

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

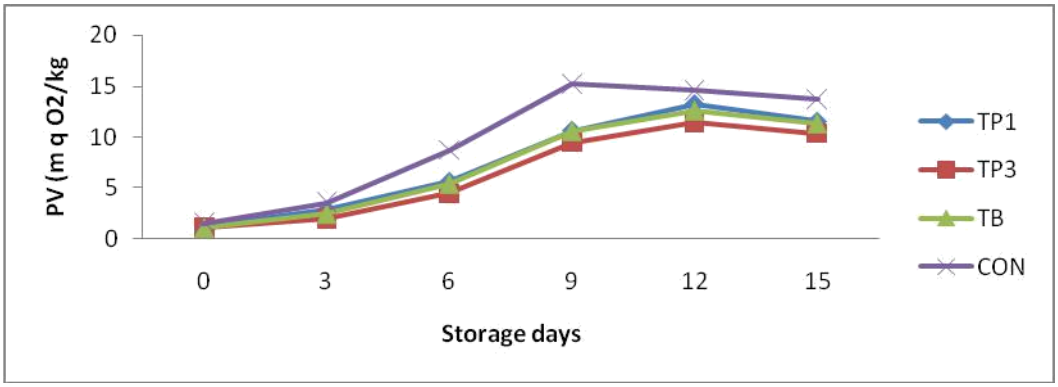


Fig.16. Changes in peroxide value of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

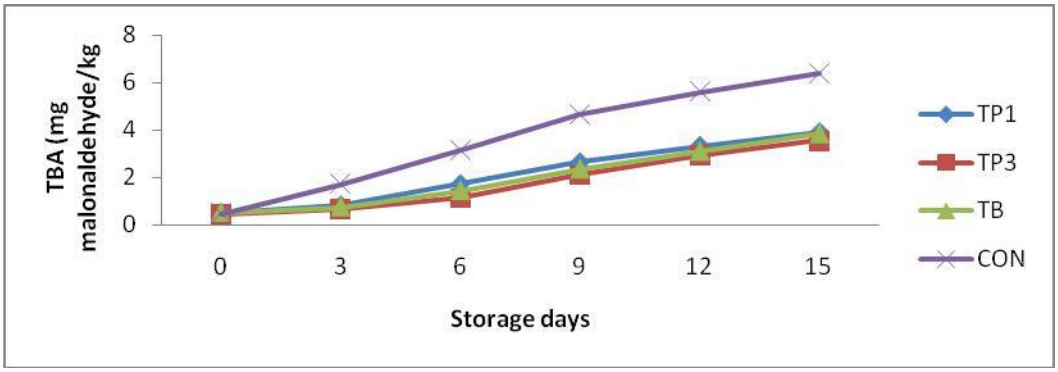


Fig.17. Changes in thiobarbituric acid of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

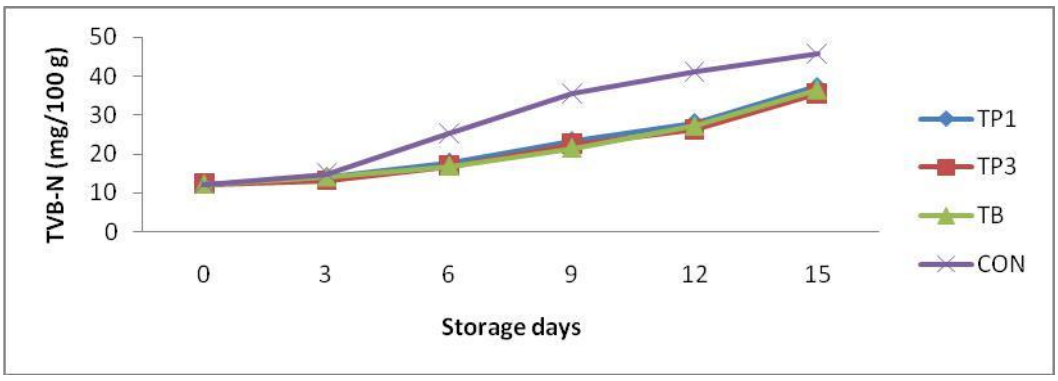


Fig.18. Changes in total volatile base nitrogen of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

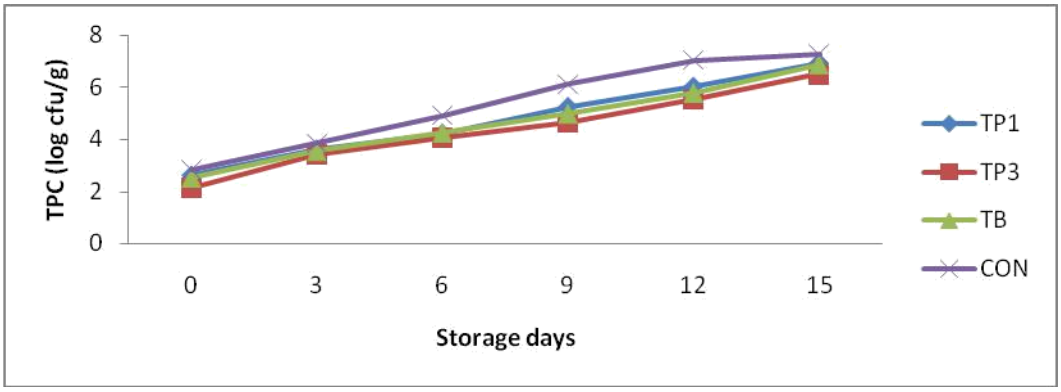


Fig.19. Changes in total plate count (TPC) of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

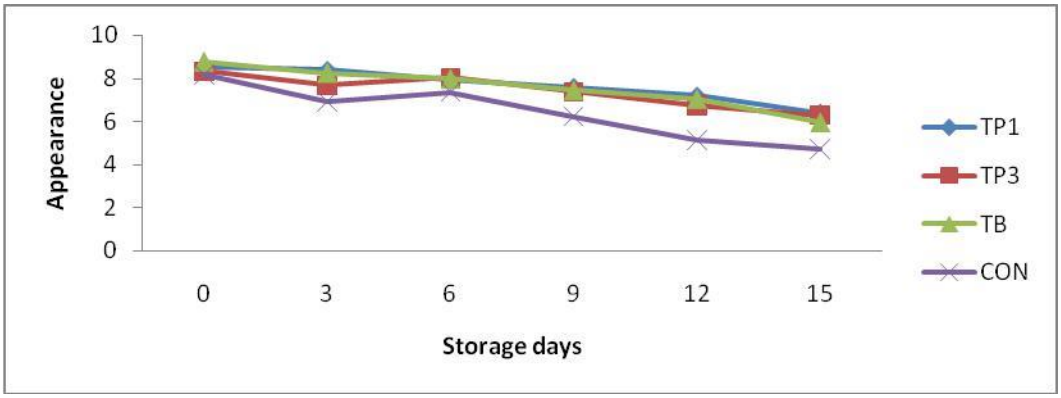


Fig.20. Changes in appearance of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

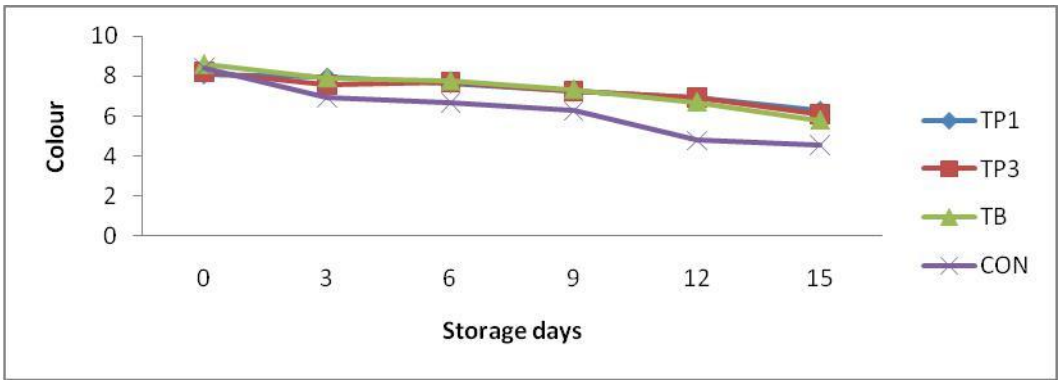


Fig.21. Changes in colour of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

4.6.3. Sensory changes during chilled storage at 4±1°C of pomegranate peel extract and BHA treated Sardine

The results of sensory score are given in Table 24 to 28. The sensory attributes were measured by general appearance, color, odours, texture and overall acceptability. The results of all sensory attributes are illustrated in Fig. 20 to 24 it shows that, initially there was change among all the treatments and control. The value decreased significantly ($p<0.05$) with storage time.

The overall acceptability values are presented in Table 28 and Fig. 24. During storage periods in control, the overall acceptability of initial value was observed as 8.28 and the final value was 3.9. In case of TP1, TP3 and TB, the initial overall acceptability was 8.64, 8.59 and 8.88 respectively and the final one was 6.48, 6.48 and 5.78 respectively. The value decreased with storage time. The overall acceptability results showed that in control the acceptance was till 9^{th} day accepted and in the treated fish TP1, TP3 and TB acceptance was up to 15^{th} day.

Table 24. Changes in appearance of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Sensory attributes	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Appearance	TP1	8.5±0.5	8.38±0.57	7.94±0.72	7.58±0.61	7.18±0.54	6.38±0.55
	TP3	8.32±0.62	7.67±0.97	8.03±0.82	7.38±0.97	6.74±0.57	6.29±0.53
	TB	8.77±0.4	8.26±0.74	7.98±0.06	7.44±0.72	7.05±0.5	5.97±0.41
	CON	8.19±0.47	6.92±1.39	7.37±0.59	6.23±0.59	5.12±0.93	4.7±0.64

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

Table 25. Changes in colour of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Sensory attributes	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Colour	TP1	8.08±0.56	7.93±0.66	7.64±0.73	7.23±0.99	6.95±0.69	6.29±0.63
	TP3	8.21±0.61	7.68±0.73	7.56±1.1	7.25±0.8	6.91±0.58	6.07±0.34
	TB	8.59±0.46	7.9±0.55	7.76±0.75	7.33±1.05	6.7±0.56	5.79±0.63
	CON	8.42±0.52	6.93±1.16	6.69±1.14	6.26±0.73	4.77±1.23	4.54±0.55

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

Table 26. Changes in odour of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Sensory attributes	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Odour	TP1	8.22±0.76	8.14±1.1	7.44±1.1	7.08±1.21	6.74±0.62	5.79±0.74
	TP3	8.23±0.59	7.86±1.1	7.36±1.02	7.18±0.93	7.06±0.58	6.33±1.04
	TB	8.61±0.47	7.74±0.62	7.39±1.08	6.76±1.04	6.44±0.7	5.17±0.66
	CON	7.67±0.67	6.72±0.94	6.47±0.82	5.35±0.53	4.28±0.28	3.97±0.97

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

Table 27. Changes in texture of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Sensory atteribute	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Texture	TP1	8.63±0.46	8±1.07	7.95±0.71	7.53±1.28	6.98±0.55	6.27±0.72
	TP3	8.57±0.72	7.99±0.95	7.8±1.12	7.61±0.92	7.19±0.43	6.68±0.71
	TB	1.13±0.20	2.56±0.16	5.43±0.20	10.6±0.16	12.6±0.16	11.36±0.32
	CON	1.56±0.09	3.6±0.28	8.7±0.16	15.23±0.12	14.56±0.46	13.7±0.35

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

Table 28. Changes in overall acceptability of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Sensory attributes	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Overall Acceptability	TP1	8.64±0.42	8.23±0.82	7.89±0.69	7.67±1.17	7.17±0.49	6.48±0.44
	TP3	8.59±0.47	7.99±1	7.85±0.8	7.63±0.88	7.24±0.48	6.48±0.39
	TB	8.88±0.19	8.08±0.59	7.96±0.8	7.5±0.94	6.9±0.45	5.78±0.4
	CON	8.28±0.5	6.82±1.48	6.71±0.84	5.86±0.81	4.7±0.67	3.9±0.72

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

Table 29. Changes in hardness1 of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Texture Profile	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Hardness 1 (kgf)	TP1	0.89±0.08	0.95±0.10	0.94±0.10	0.93±0.17	0.60±0.07	0.59±0.06
	TP3	1.25 ±0. 46	0.91±0.05	1.17±0.04	1.20±0.50	0.64±0.25	0.60±0.20
	TB	1.15±0.04	0.88±0.08	1.02±0.00	1.01±0.00	0.64±0.07	0.59±0.04
	CON	0.83±0.14	0.86±0.16	0.85±0.24	0.85±0.12	0.56±0.04	0.54±0.04

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

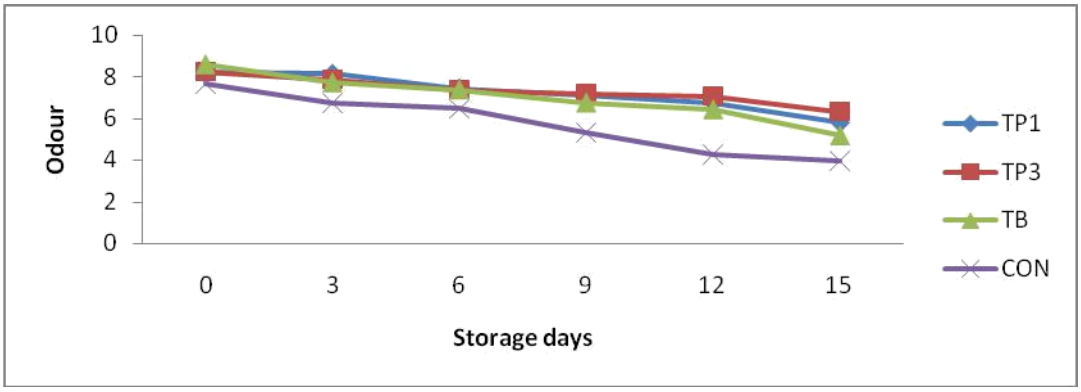


Fig.22. Changes in odour of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

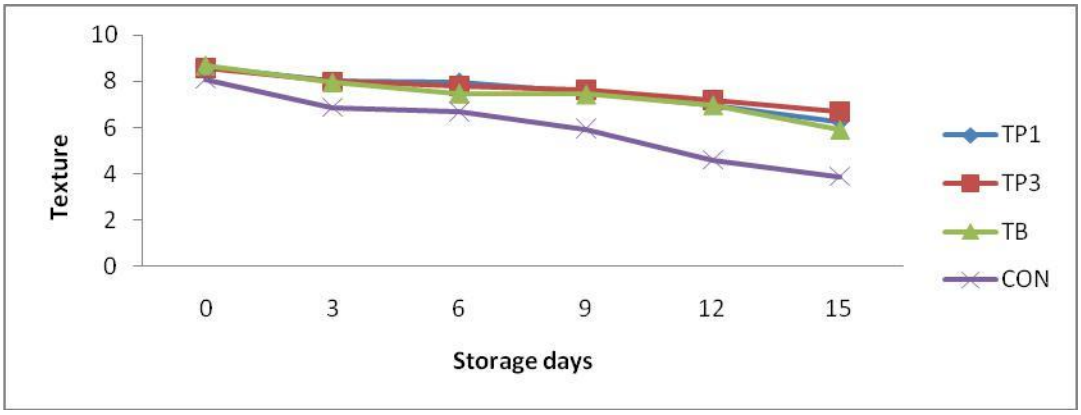


Fig.23. Changes in texture of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

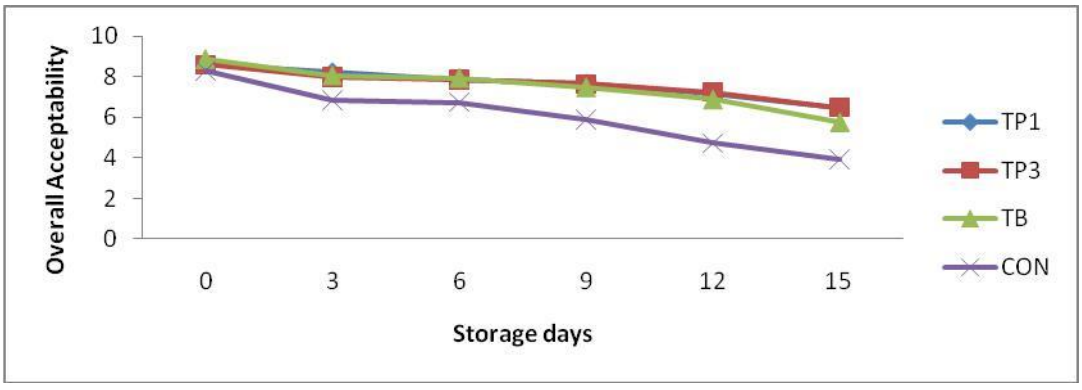


Fig.24. Changes in overall acceptability of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

4.6.4. Texture profile analysis (TPA) during chilled storage at 4±1°C of pomegranate peel extract and BHA treated sardine

During the 15 days of storage, some indices of treated and control fish showed decreased trends, likely hardness1, hardness 2, springiness, cohesiveness, springiness and chewiness. The difference for changing trend of all factors among all the treatment sample and control are presented in Table 29 to 33 and Fig. 25 to 29.

4.6.4.1. Changes in hardness1 of sardine treated pomegranate peel extract and BHA during chilled storage condition at 4±1°C

During the chilled storage TP1, TP3, TB and control, hardness1 values increased and thereafter decreased. In case of TP1, TP3, TB and CON, the hardness 1 initial value was 0.89, 1.25, 1.15 and 0.83 kgf and final value was 0.59, 0.60, 0.59 and 0.54 kgf respectively. The results showed that the TP3 value is better compared to TP1, TB and control. The results are presented in Table 29 and Fig 25.

4.6.4.2. Changes in hardness2 of sardine treated pomegranate peel extract and BHA during chilled storage condition at 4±1°C

The hardness 2 value for all treated and control samples are presented in Table 30 and Fig 26. In case of control the hardness 2 initial value was 0.64 and final value was 0.42, values decreased till the end of the storage days. For TP1, TP3 and TB, the hardness 2 initial values were 0.70, 0.94 and 0.92 kgf and the final recorded values were 0.50, 0.51 and 0.48 which decreased with storage time. Among all treatment the TP3 showed better results followed by TP1<TB<Control.

4.6.4.3. Changes in cohesiveness of sardine treated pomegranate peel extract and BHA during chilled storage condition at 4±1°C

The cohesiveness results are presented in Table 31 and Fig. 27. In case of control, the cohesiveness initial value was 0.29 and final one was 0.35 kgf. Similar results were shown for TP1, TP3 and TB, the cohesiveness initially was 0.30, 0.35 and 0.31 and on 15^{th} day was 0.36, 0.37 and 0.37 respectively. The values were following decreasing trend with increase in storage period.

4.6.4.4. Changes in springiness of sardine treated pomegranate peel extract and BHA during chilled storage condition at 4±1°C

The springiness initial value for control was 1.41 mm and the final 1.44 mm. After 3^{rd} day the value was constant till the end of the storage period. In case of TP1, TP3 and TB, the springiness initial value was 1.52.1.68 and 1.65 mm the final value was 1.47, 1.57 and 1.56 mm respectively. The value decreased with storage time is presented in Table 32 and Fig.28.

4.6.4.5. Changes in Chewiness of sardine treated pomegranate peel extract and BHA during chilled storage condition at 4±1°C

The chewiness values are presented in Table 33 and Fig.29. For control, the chewiness initial value was 0.34 kgf and final value was 0.27 kgf. In case of TP1, TP3 and TB the initial value was observed as 0.38, 0.69 and 0.57 kgf and the final value was 0.31, 0.34 and 0.34 kgf respectively. There values were also decreasing with storage time.

Table 30. Changes in hardness 2 of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Texture Profile	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Hardness 2 (kgf)	TP1	0.70±0.19	0.76±0.08	0.78±0.02	0.78±0.13	0.53±0.04	0.50±0.04
	TP3	0.94±0.05	0.75±0.16	0.80±0.14	0.83±0.17	0.55±0.15	0.51±0.30
	TB	0.92±0.01	0.69±0.06	0.76±0.03	0.82±0.00	0.52±0.04	0.48±0.05
	CON	0.64±0.11	0.75±0.10	0.72±0.03	0.70±0.09	0.46±0.05	0.42±0.03

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON – control

Table 31. Changes in cohesiveness of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Texture Profile	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Cohesiveness	TP1	0.30±0.02	0.31±0.01	0.39±0.06	0.37±0.01	0.37±0.03	0.36±0.02
	TP3	0.35±0.08	0.32±0.06	0.35±0.08	0.34±0.01	0.37±0.07	0.37±0.03
	TB	0.31±0.00	0.30±0.01	0.33±0.03	0.32±0.01	0.38±0.02	0.37±0.00
	CON	0.29±0.01	0.29±0.00	0.33±0.026	0.33±0.01	0.36±0.01	0.35±0.03

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

Table 32. Changes in springiness of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Texture profile	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Springiness (mm)	TP1	1.52±0.03	1.49±0.02	1.59±0.00	1.60±0.00	1.48±0.09	1.47±0.06
	TP3	1.68±0.12	1.51±0.12	1.57±0.16	1.55±0.06	1.60±0.19	1.57±0.26
	TB	1.65±0.01	1.50±0.00	1.51±0.01	1.56±0.00	1.56±0.04	1.56±0.01
	CON	1.41±0.05	1.46±0.04	1.45±0.01	1.44±0.02	1.44±0.07	1.44±0.09

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

Table 33. Changes in springiness of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

Texture profile	Treatments	0th day	3rd day	6th day	9th day	12th day	15th day
Chewiness (kgf)	TP1	0.38±0.08	0.49±0.04	0.52±0.08	0.58±0.10	0.35±0.09	0.31±0.07
	TP3	0.69±0.27	0.42±0.22	0.51±0.00	0.53±0.08	0.39±0.20	0.34±0.18
	TB	0.57±0.04	0.40±0.01	0.51±0.00	0.52±0.02	0.39±0.00	0.34±0.01
	CON	0.34±0.057	0.39±0.09	0.37±0.04	0.44±0.04	0.32±0.08	0.27±0.05

Note: TP1 and TP3 – pomegranate peel extract 1% and 3%, TB – BHA , CON - control

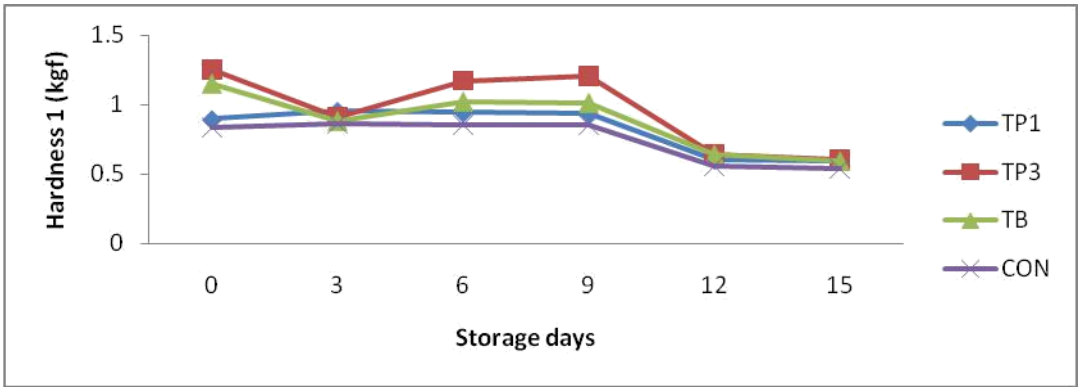


Fig.25. Changes in hardness1 of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

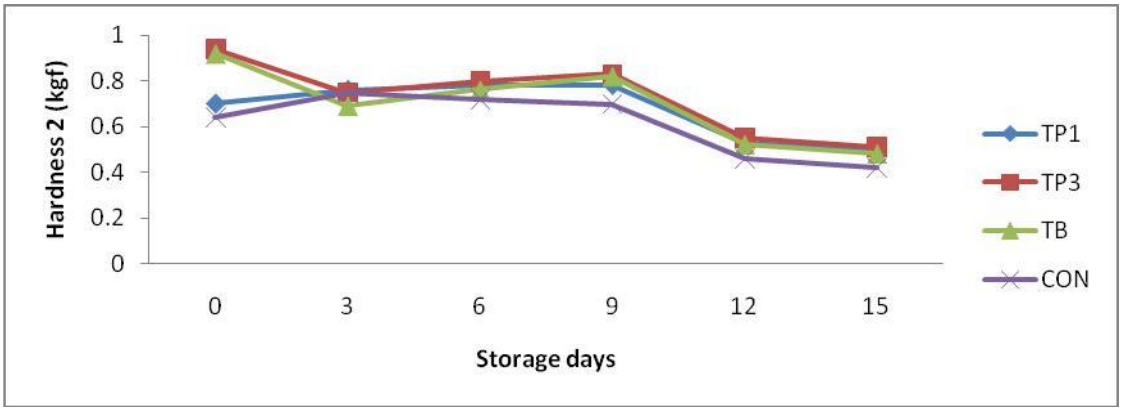


Fig.26. Changes in hardness2 of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

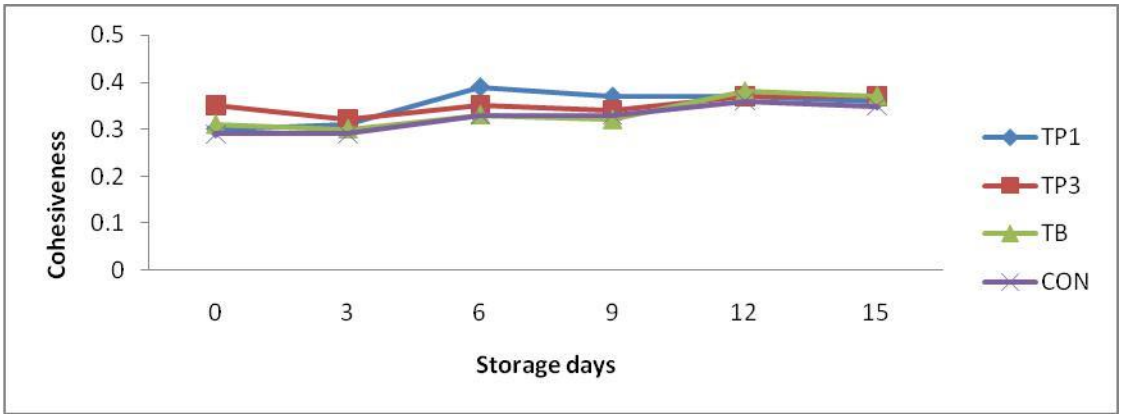


Fig.27. Changes in cohesiveness of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

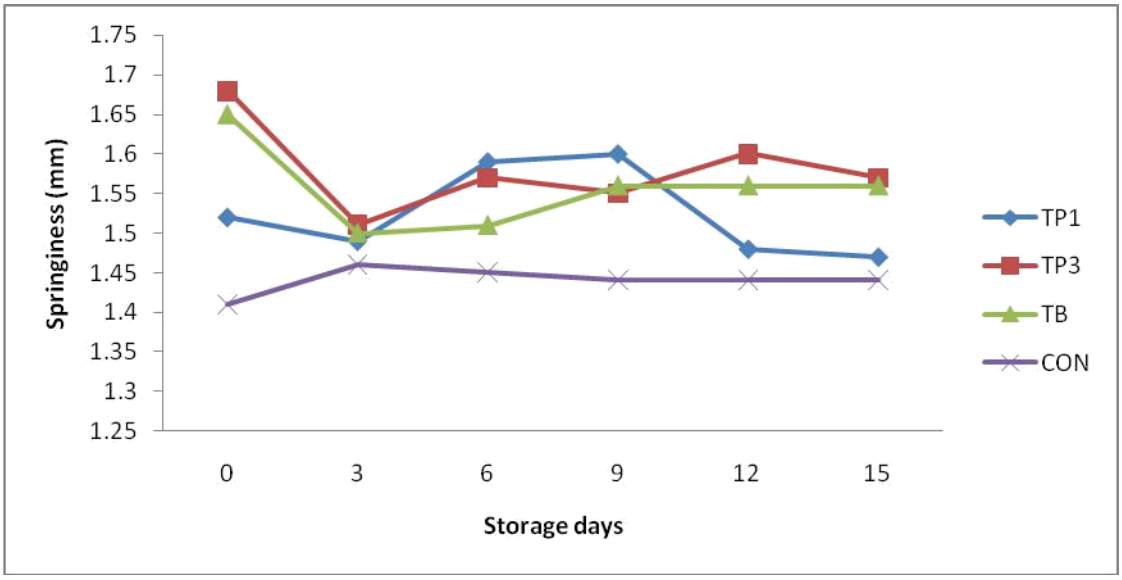


Fig.28. Changes in springiness of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

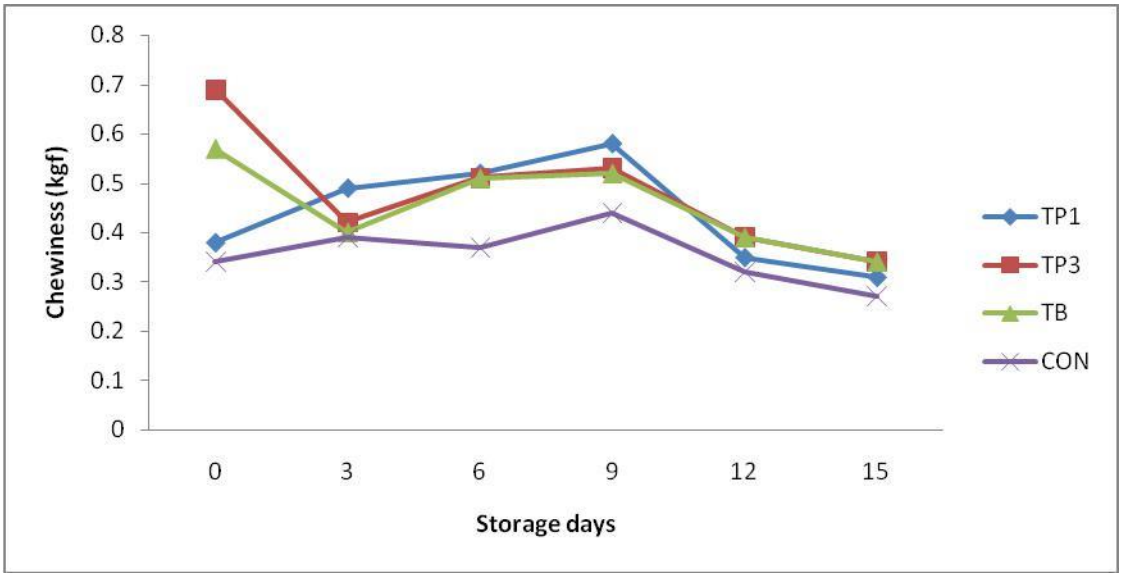


Fig.29. Changes in springiness of sardine treated with pomegranate peel extract and BHA during chilled storage condition at 4±1°C

V. DISCUSSIONS

5.1. Extraction yield

In the present study, the extraction was done using methanol. PPE (pomegranate peel extract) the highest extraction yield of 23.74 %, OPE (Orange peel extract) 20.06 % and PAPE (pineapple peel extract) of 17.11% respectively. The extraction yield of pomegranate was coherent to the work reported by Singh and Immanuel (2014) and the difference occurred could be due to the solvent used for extraction of natural compounds. Rahman (2006) reported 11.24±0.81 % yield when citrus peel was extracted using ethanol as solvent. The extraction yield of OPE was higher which can be attributed to the species of orange used for the experiment or the environmental condition, Similar result extraction yield of PAPE was reported by Alejandra and Emperatriz (2011).

5.2. Total phenolic content and total flavonoid content

In the present study, TPC of the pomegranate peel extract (PPE), orange peel extract (OPE), pineapple peel extract (PAPE) and combined fruit peel extracts (CFPE) were estimated by the folin-ciocalteu methods The TPC values of the PPE was higher (226 mg/g GAE) than CFPE (152 mg/g GAE), OPE (141.66 mg/g GAE) and PAPE (137 mg/g GAE). The TFC (total flavonoid content) values of the PAPE was higher 189.33 mg/g QAE than PPE i.e.177.33 mg/g QAE, CFPE 111.33 mg/g QAE and OPE 88.66 mg/g QAE respectively. According to Shinde et al., (2015) the phenolic content in pomegranate peel extract (PPE) was observed to be 212±20.55

mg TAE/g. Similar result were reported by Yerlikaya et al. (2010) where total phenolic content of pomegranate peel extract was 249±17.2 mg TAE/g. Uchoi et al. (2017) reported that the pineapple have total phenolic and total flavonoids content of 131 mg GAE 100g-1 and 211.2 mg QE 100g-1 respectively. It has been shown that orange and pomegranate peel is a good raw material for producing natural antioxidants because of its high content of antioxidants (Anagnostopoulou et al., 2005; Qu et al., 2010). The total phenolic content of citrus peel extracts was much higher than those reported by Ghasemi et al. (2009) due to the variation in the method of extraction followed and the species of orange peel used.

5.3. DPPH radical scavenging activities

Several free radicals, such as OH^-, O_2^-, LOO^- · having different reactivities are formed during lipid oxidation. Antioxidants are able to scavenge these free radicals by donating an H atom. Relatively stable DPPH radical has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity (Jao and Ko 2002). PPE extracts showed higher radical scavenging activity at the concentration of 4 µg/mL with the inhibitory activity of 88.46% and for BHT 91.87% while CFPE, PAPE and BHT showed the radical scavenging activity at a concentration of 6 µg/mL i.e. 83.09%, 76.85%, 89.54% and OPE showed the radical scavenging activity at a concentration of 8 µg/mL i.e. 81.53 respectively. Among all the fruit peel extracts PPE showed the higher radical scavenging activity followed by CFPE, OPE and PAPE. Pal et al. (2017a) reported that the PPE extract has the radical scavenging activity of 92.38% at the concentration of 35µg/mL but at the same concentration

BHA showed inhibition of 93.59 % which is almost equivalent to PPE. The OPE showed the radical scavenging activity 85.68 % at a higher concentration of 700 µg/mL. Uchio et al. (2017) reported that the PAPE to exhibit maximum DPPH radical scavenging activity of 81.75% at 2 mg-1ml. Saoni (2016) reported that the radical scavenging ability of PPE to be 12.5±1.5% at 50 mg/L, 22.7±0.9% for 100 mg/L, 63.7±1.2% for 250 mg/L, 73.6±1.9% for 500 mg/L and 74.5±0.S% for 1000 mg/L concentration.

5.4. Proximate composition of fresh sardine (*Sardinella gibbosa)*

The proximate composition of sardine. In the present study, the fresh sardine (*Sardinella gibbosa*) had moisture, protein, fat and ash content of (% wet weight basis) as 78.78%, 19.18%, 2.66% and 1.45% respectively. Similar proximate composition of *S. gibbosa* (moisture 73.10%, protein 22.32%, fat 4.3% and ash 7.87%) has been reported by Annathai et al, (2014). However, the protein content of sardines are available in the Pakistan coasts ranged from 14.11 to 18.78% (Munshi et al*.,* 2005). Hale (1981) reported the similar value of fat content (2.42%) in Spanish sardine and he also stated that the proximate composition of sardine did vary with the season, nature of food availability, migration and reproductive cycle.

5.5. Standardization of application of fruit peel and their combined peel extracts on sardine under chilled storage condition at 4±1˚C

5.5.1. Biochemical Changes

5.5.1.1. Changes in PV

Changes in the peroxide value of sardine treated with different concentration of PPE and BHA. The PV found in initial day for TP1 was (1.46), TP3

(1.16), OP1 (1.9), OP3 (1.4), TPA1 (1.83), TPA3 (1.83), TCF1 (1.6), TCF3 (1.33), TB (1.2) and TT (1.3) meq/kg. PV of all the samples was significantly ($P<0.05$) increasing with storage time till 3rd day. The sample taken as control which was not treated with any antioxidant had increased its PV at the maximum up to 3.56 at the 3rd day and it was the highest value among all the samples. A significant difference observed between control and all the treatment samples ($P<0.05$). The PV recorded in the sample treated at the concentration of 3% (TP3) showed significantly ($P<0.05$) lower value than other samples TP1,TPA1,TPA3 , TO1, TO3, TCF1, TCF3, TB and TT throughout the storage period. Among TB and TT, the TB value was lower. Tarkhasi (2016) reported that such changes in peroxide value of edible Coating pomegranate peel extract on silver carp (Hypophthalmichthys molitrix) fillet during refrigerated storage, the initial PV (meq peroxide/kg fish sample) in the control sample was 1 and increased to 15.33 at the end of storage. No significantly difference ($P>0.05$) was observed throughout first days of the storage among the different sample. PV content significantly increased ($P<0.05$) in all treatments throughout the refrigerator storage, indicating that lipid deterioration continued under the storage temperature conditions. At the end of the storage time significant differences ($P>0.05$) were observed in the PV between the control (15.33) and each of T1 (14.23) and T2 (12.60) samples. PV of T1 sample was also significantly higher than of T2 samples. Storage time has a significant effect on the PV for each of control and treated samples ($P<0.05$). The higher antioxidant capacity of pomegranate peel is related to the presence of phenolic compounds, especially

elagic acid and punicalagin that can act as free radical scavengers during the oxidation in the food system (Sarkhosh et al., 2007).

5.5.1.2. Changes in TBA

Changes in the TBA value of sardine fish oil treated with different fruit peel , the combined , BHA and BHT. In case TP1, TP3, TO1, TO3, TPA1, TPA3, TCF1, TCF3, TT and TB, the initial TBA value was 0.69, 0.56, 0.70, 0.64, 0.71, 0.69, 0.70, 0.68, 0.58 and 0.58 mg MA/kg of fat respectively, it increased throughout the storage period for 3 days. The control sample without any antioxidant showed significant ($P<0.05$) increase in TBA value than the samples treated with different fruit peel, the combined one, BHA and BHT. The highest TBA value recorded was 1.61 mg MA/kg for control sample at the day 3. The sample TP1, TP3, TO1, TO3, TPA1, TPA3 , TCF1, TCF3, TT and TB had the TBARS value of 0.78, 0.64, 0.80, 0.75, 0.81 0.77, 0.79, 0.74, 0.67and 0.71 mg MDA/kg at the day 3 and this result showed that there is significant ($P<0.05$) difference between l the treated samples. This proves that the TP3 has high potential to act as an effective natural antioxidant instead of synthetic antioxidants in arresting oxidation in sardine fish. Ozen & Soyer et al. (2018) also reported such changes in TBARS values of mackerel mince during frozen storage. Secondary oxidation products presented low values at the beginning of the storage and gradually increased for all groups throughout storage. The level of TBARS in control samples increased speedily from 2.80 to 8.25 mg MDA/ kg during preliminary 3 month of storage, then increased up to 14.25 mg MDA/kg at the end of storage. Whereas pre-treated samples showed a slight increase in TBARS values from 2.34 to 7.57 mg MDA/kg throughout storage study. Ozgul et al. (2010) was

reported by stating the treatments with rosemary extract to the sardine fillets significantly reduced the formation of TBARS value.

5.5.1.3. Changes in TVB-N

In the present study TVB-N was 13.06 mg % at the beginning of the storage in TP1, TO1, TO3, TPA3, TCF1 and TCF3. The TVB- N value in TP3, TB and TT was 12.13 mg %. The TVB-N content was observed to increase progressively in all treated samples. However, the increment of TVB-N content was rapid in control 14.93 to 16.8 at the end of 3 days storage. The lower TVB-N values were recorded in TP3, TB and TT samples during storage.These findings are in agreement with Unalan et al., (2011). The TVB-N values for these treatments significantly increased ($P < 0.05$) during the chilled storage and are below limit level at the end of chilled storage. The results of TVB-N of all treated sample were significantly increased with increase in storage period and highest a increasing trend was recorded in case of control sample. The increasing trend of TVB-N value fish ham stored at the ambient storage was also reported by Rajalakshmi (2014).

5.5.2. Microbiological changes

Total plate count of TP1, TP3, TO1, TO3, TPA1, TPA3, TCF1, TCF3, TT and TB samples stored in chilled storage were comparatively lower than that of control samples. In TP1, TP3, TO1, TO3, TPA1, TPA3, TCF1, TCF3, TT and TB, the initial TPC value was 3.4, 3.2, 3.6, 3.43, 3.6, 3.46, 3.5, 3.36, 3.06 and 3.1 cfu/g respectively. TPC of all the samples significantly ($P<0.05$) increased with storage time till 3rd day. In the control the TPC reached 3.93 on the 3rd day and it was the

highest value among all the samples. The TP3 Showed lower value compare to other samples. These findings are in agreement with Ibrahium (2010). The phenolic compounds such as carvacrol, eugenol, thymol, green tea extract, rosemary extract, grape fruit seed extract and lemon extract control the microbiological spoilage in packed fresh cod hamburgers and were studied by Corbo et al. (2009).

5.5.3. Sensory analysis

In treated and control sardine fish there was no significance difference ($p<0.05$) among all the treatments samples. All the sensory attributes (appearances, colour, odour texture and overall acceptability) decreased but were at acceptable level. As far as overall acceptability scores are concerned in control, the initial value of was 7.85 and the final value was 6.69 observed. The value was decreased with storage time. In case of TP1, TP3, TO1, TO3, TPA1 TPA3, TCF1, TT and TB, the value was significantly decreased (<0.05) with storage time. The initial value showed was 8.66, 8.01, 8.21, 8.08, 8.25, 8.48, 8.46, 8.55, 8.29, and 8.24 observed and the final one was 7.78, 7.39, 7.86, 7.65, 7.87, 7.73, 7.87, 7.43, 7.34, and 7.17 respectively. The results showed that among all treatments the TP1 and TCF1 has more overall acceptability followed by TO1<TP1<TPA3<TO3<TCF<TP3<TB< TT. Ozogul et al. (2010) reported that hedonic scale from 9 to 1 was used to evaluate sardine treated with rosemary extract, such as sensory scores on sardine fillets (C, R1 and R2) reached the limits of acceptance 13, 17 and 20 days of storage, respectively. Papadopoulos et al. (2003) has reported a shelf life of 13 and 8 days respectively for whole ungutted and gutted sea bass samples during ice storage by used 9- point headonic scale.

5.5.4. Texture analysis

The hardness 1 in control the initial value was 0.72 after that the value was constant till the end of 3 days of storage period. In case of TPA1 and TT, the hardness 1 initial value was 0.75 and 0.67 it increased up to two days and then the value was significance decreased ($p<0.05$). In case of TP1, TP3, TO1, TO3, TPA3, TCF1, TCF3 and TB samples, the hardness-1 initial value was 1.01, 1.11, 0.87, 0.94, 1.00, 0.86, 0.78, and 0.67 kgf respectively and the value decreased with storage time. There was a significance difference ($p<0.05$) in the entire sample. For hardness-2 in TP1, TO1, TO3, TPA1,TPA3, TCF1 and TCF3 samples, the initial value was 0.87, 0.63, 0.72, 0.74, 0.61, 0.72, and 0.64 kgf respectively after that the value decreased . In case of TPA3, the initial value of hardness 2 was 0.59 after that the values decreased and again increased at the end of 3 days with a significant difference ($p<0.05$). In case of TP3, TB, TT, and control the initial value was progressily increased to 0.63, 0.73, 0.55 and 0.03 kgf respectively after that the values were decreased with storage time. For cohesiveness of TP1, TO1, TPA1, TPA3 and TCF1 samples, the cohesiveness initial value was 0.33, 0.30, 0.32, 0.32 and 0.28 respectively, the value decreased with storage time. In case of TPA3, TO3, TCF3, TB, TT and control, the initial value of cohesiveness was increased to 0.31, 0.31, 0.28, 0.27, 0.27, 0.29 and 0.27 respectively after that the values having significant decrease ($p<0.05$) at the end of 3 days of storage period. The springiness of TP1, TP3 and TPA1 samples initially was found to be 1.66, 1.66 and 1.57 mm respectively. The value increased with storage time. In case of TO1, the initial value of springiness was 1.47 and it was constant at the end of storage time. In case of

TCF1, TCF3, TB, TT and control sample, the springiness initial value was 1.55, 1.48, 1.49, 1.56 and 1.46 mm respectively and the value decreased significantly in all the sample ($p<0.05$).The initial value of chewiness for TP1, TP3, TO1, TO3, TPA3 and TCF3 samples, was 0.59, 0.62, 0.40, 0.44, 0.58 and 0.36 kgf respectively, the value decreased with storage time. In case of TPA1, TCF1, TB, TT and control, the initial value of chewiness was increased 0.36, 0.25, 0.41, 0.34 and 0.28 kgf respectively, then decrease at the end of 3 day of storage study. The value of texture profile vary may be the species varies. Liu et al., (2013) found that the texture profile of grass carp fillets stored at −3°C and 0°C decreased sharply within the first 3 days. The decrease in texture quality of fish stored at 4°C may be due to protein changes during the storage process (Ayala et al., 2010). Viji et al., 2015 reported decreases in hardness 1, hardness 2, cohesiveness, and springiness and chewiness values after first day might be attributed to the weakening of connective tissue of fish muscle during storage.

5.9. Storage study of pomegranate peel extract and BHA treated sardine under chilled storage condition at 4±1˚C

5.9.1. Biochemical changes

5.9.1.1. Peroxide Value

In TP1, TP3, TB samples of the PV found on the initial day was 1.23, 1.06, 1.13 and 1.56 meq/kg. PV of all the samples was significantly ($P<0.05$) increasing with storage time till ninth day for control and for treated samples till 12^{th} day and after 9 days for control and 12^{th} day for treated sample PV was

decreasing with increasing storage time. The sample taken as control increased to maximum upto 14.56 meq/kg at the ninth day and it was the highest value among all the samples. The PV value of the sample with TB increased to 12.6 meq/kg at 12^{th} day and then decreased to 11.36 meq/kg on the 15^{th} day. The samples TP1 and TP3 increased to 13.23 and 11.43 meq/kg on the 12^{th} day and decreased to 11.53 and 10.33 meq/kg on the 15^{th} day respectively. There was a significant difference ($P<0.05$) between control and all the treatment samples TP1, TP3 and TB. The PV recorded in the sample TP3 showed significantly ($P<0.05$) lower value than the TP1 and TB throughout the storage period. Hasani et al. (2016) reported about the changes in PV of common carp fillets during the storage period. The PV values of samples significantly increased ($P < 0.05$) during the storage time, until day 12, and then decreased gradually until the end of the storage time. The peroxide value provides a measure of the degree of lipid oxidation and indicates the amount of oxidized substances. These are usually hydro peroxides, which produce iodine from potassium iodide under special conditions. Hydro peroxides are the primary products of autoxidation, which themselves are odorless. However, their decay leads to the formation of a wide range of carbonyl compounds, hydrocarbons, furans and other products that contribute to the rancid taste of decaying food (Singh RP et al., 2002). Pazos et al. (2005) reported that the citric acid and grape seed extract solution coating was effective in reducing the formation of primary lipid oxidation products in horse mackerel fillets stored at -20 ^{o}C. The higher reduction in TBARS value of the sample treated with PPE could be due to its high amount of phenol accounting for its strong antioxidant ability (Chen

et al. 1991).

5.9.1.2. Changes in Thiobarbituric Acid

The TP1, TP3 and control initial TBA value was 0.48, 0.43, 0.46 and 0.51 mg MDA/kg of fat. The TBA shows increasing trend throughout the storage period for 15 days. The control sample without any antioxidant showed significant ($P<0.05$) increase in TBA value than the samples TP1, TP3 and TB. The highest TBA value recorded was 6.37 mg MDA/kg for control sample at the day 15. The sample TP1 and TP3 had the TBA value of 3.92 and 3.55 mg MDA/kg and TB sample showed 3.83 mg MDA/kg at the day 15 and this result showed that there is no significant ($P>0.05$) difference between the samples TP1, TP3 and TB. This proves that the TP3 higher concentration have the potential to act as an effective natural antioxidant instead of synthetic antioxidants in arresting oxidation in sardine fish. Pal et al. (2017b) was reported that the TBARS values of sample (Indian mackerel) treated with PPE extracts stored at 4°C over 8 days. The TBARS value of all treated sample significantly increased ($p<0.05$) with increase in storage time. The sample treated at a concentration of 2000 ppm showed lesser formation of secondary oxidation products compared to the sample treated with the control, 1000 and 1500 ppm. Lower formation of TBARS contents in the sample treated with 2000 ppm can attribute due to high concentration of phenolic contents. Li et al., (2006) showed that pomegranate peel extract had markedly higher antioxidant capacity than the pulp extract in scavenging or preventive capacity against superoxide anions and hydroxyl and peroxyl radicals. Devatkal and Naveena (2009) A similar finding related antioxidant effect of PPE had been reported that addition of PPE extract reduce

autoxidation in treated sample, and also reported that presence negative correlation between total phenolic and TBA values. They showed that TBA value increased gradually and total phenolic decreased with increase in storage period.

5.9.1.3. Changes in Total volatile base nitrogen

In the present study TVB-N was 12.13 mg % initial for TP1, TP3, TB and control. The TVB-N content was observed to increase progressively in all treated samples. However, the increment of TVB-N content was rapid in control 45.73 mg% at the 15^{th} day of storage. The lower TVB-N values were recorded in TP3, TB and TP1 samples 35.46, 36.4 and 37.33 mg% respectively at 15^{th} day. The TVB-N values for these treatments significantly increased ($P < 0.05$) during the chilled storage and for TP1, TP3 and TB in acceptable limit level till 12^{th} day and for control till 9^{th} day of chilled storage. TVB-N was 7.12±0.58 mg % at the beginning of the storage in PPE treated as well as GTE treated samples. Shinde and Patange (2015) reported that the TVB-N content was observed to increase progressively in both PPE & GTE treated mackerel samples. However, the increment of TVB-N content was rapid in GTE treated samples than PPE treated samples. The lower TVB-N values were recorded in PPE treated sample during storage. The values in treated samples below or close to limit level indicated that the increase in TVB-N value was slow in the PPE treated samples as compared to GTE treated samples. The TVB-N value, which is mainly composed of ammonia and primary, secondary and tertiary amines, is widely used as an indicator of meat deterioration. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Ojagh et al., 2010). The lowest increase of TVB-N was recorded in the ham treated with PPE extract,

because PPE contain bioactive compounds like peel polyphenols, tannins, flavonoids and anthocyanins bearing antimicrobial activity as reported by the Khan and Hanee (2011).

5.9.2. Microbiological Changes

Total plate count of TP1, TP3 and TB samples stored in chilled storage were comparatively lower than that of control samples. In TP1, TP3, TB, and control the initial TPC value was 2.63, 2.13, 2.5 and 2.8 log cfu/g. TPC of all the samples was significantly ($P<0.05$) increasing with storage time till 15 days. The sample of TP1, TP3 and TB, the TPC value was 6.93, 6.5 and 6.86 cfu/g at the 15 days and the sample taken as control which was not treated with any antioxidant had increased its TPC at the maximum up to 7.26 cfu/g at the 15 days and it was the highest value among all the samples (Viji et al., 2015) reported the changes in APC of mackerel throughout storage. The fresh fish showed a count 4.17 10 cfu/g, demonstrating its good quality. There were no considerable differences among the APC of different samples up to 6 days of storage. On and after 9th day, the control group showed 1–1.2 log higher counts than ME group. The counts of CE treated samples were 0.2–0.5 log lesser than that of control after 9 days during storage. At the sensory rejection day, the APC reached up to 7.13, 7.01 and 7.33 log10 cfu/g, respectively for control, CE and ME groups. (Qi et al., 2004) reported that chitosan nanoparticles exhibit higher antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* than chitosan on account of the special character of the nanoparticles, likely the nanoparticle‘s larger surface area and higher affinity with bacteria cells, which yields a quantum-size effect. The

least APC value in samples treated with PPE extract might be also due to the evaluated antimicrobial characteristics of pomegranate peel extract and its inhibition effect against gram positive and gram negative bacteria as reported by Kanatt et al. (2008).

5.9.3. Sensory analysis

The sensory attributes were measured by general appearance, color, odours, texture and overall acceptability. The sensory score value was decreased significantly ($p<0.05$) with storage time. During storage periods in control, the overall acceptability was 8.28 and final was 3.9. In case of TP1, TP3 and TB, the initial overall acceptability was 8.64, 8.59 and 8.88 and the final was 6.48, 6.48 and 5.78 respectively. The value significantly decreased ($p<0.05$ with storage time. The overall acceptability results showed that the control was accepted up to 9^{th} day and the treated fish TP1, TP3 and TB were upto 15^{th} day. Li et al., (2012) observed a significant decrease in the overall acceptability after 8 days of storage of untreated large yellow croaker, which agreed well with a concomitant shift in bacterial counts. Similarly, in this study, sensory evaluation results appeared to be correlated to microbial and chemical value analyses. Ababouch et al. (1996) reported that the keeping time of sardines (*Sardina pilchardus*) varied between 21 and 27 h (average 23 h) for fish stored at ambient and from 8-11 days (average 9.5 days) in ice. Surendran and Iyer (1985) reported that the shelf life of pearl spot (Etroplus suratensis) in ice was 8-10 days. The antioxidant and antimicrobial activity by herbal extracts have been shown to prolong the products shelf life while maintaining their quality. The combined treatment of both turmeric and shallot extracts enhanced the

beneficial effects on texture, odor, color, and overall acceptability of the fish in final days (Pezeshk et al., 2011).

5.9.4. Texture profile analysis

During chilled storage Hardness-1 for TP1, TP3 TB and control, was increased and after that the value decreased with storage time. In case of TP1, TP3, TB and control, the hardness 1 initial value was 0.89, 1.25, 1.15 and 0.83 kgf and final value was 0.59, 0.60, 0.59 and 0.54 kgf respectively. The results showed that the TP3 value is better compared to TP1, TB and control. For Hardness-2 all the treated and control samples results is presented Table 4.29 and Fig.4. 27. In case of control the hardness 2 initial value was 0.64 and final value was 0.42 kgf , value decreased with end of the storage days. In case of TP1, TP3 and TB, the hardness-2 initial values were 0.70, 0.94 and 0.92 and the final value was 0.50, 0.51 and 0.48 kgf significantly decreased ($p<0.05$) with storage time. Among all treatment the TP3 showed better results followed by TP1<TB<control. In case of control, the cohesiveness of initial value was 0.29 and final value at the end of the storage was 0.35. Similar results shown for TP1, TP3 and TB, the cohesiveness initial value was 0.30, 0.35 and 0.31 and final value was 0.36, 0.37 and 0.37 respectively. There was significantly decrease ($p<0.05$) with storage time. The springiness in control, 1.41 and final value reached was 1.44 mm. In case of TP1, TP3 and TB, the initial value was 1.52.1.68 and 1.65 mm the final value was 1.47.1.57 and 1.56 mm respectively. There was significance decrease ($p<0.05$) with storage time. In case of control , the chewiness initial value showed was 0.34 and final value was 0.27 kgf. In case of TP1, TP3 and TB the initial value was observed 0.38, 0.69 and 0.57 and the final

value was 0.31,0.34 and 0.34 kgf respectively and the value significantly decreased ($p<0.05$) with storage time. Yi Sun et al. (2018) reported that during shelf life analysis of mandrine fish, some indices of raw and cooked fish fillet showed decreasing trends, such as in hardness, springiness, gumminess, and chewiness. The cohesiveness of raw fish fillet did not show a significant change throughout the storage period. Conversely, the cohesiveness of cooked fish fillet increased in the first 2 days and then decreased gradually during further storage. Gao et al. (2014) discovered that the average values for hardness, gumminess, chewiness, and springiness of pompano fillets displayed significant decreases throughout 15 days of storage at 4 ± 1°C. This is in agreement with the result of Manju et al. (2007) who observed a slight reduction in hardness, springiness chewiness and cohesiveness value of pearl spot during ice storage.

VI. SUMMARY AND CONCLUSIONS

The seafood represents an excellent option as a major source of nutrients. The consumption of fish is compatible with the reduction of various diseases, like Coronary heart disease, hypertension, cancer, obesity, iron deficiency, protein deficiency, osteoporosis and arthritis leading to contemporary health problems for which fish provides a number of nutritional advantages and some therapeutic benefits. However, due to high content of unsaturated lipids in the fish and fishery products it is highly prone to oxidative deterioration. However, antioxidants can be used to protect the quality of fish and fishery products by preventing oxidative deterioration. Hence, the antioxidants play an important role in the preparation, packaging, and storage of fish and fishery products. The most common antioxidants used in the food industry are BHA, BHT, and TBHQ. However, the use of some antioxidants such as BHA in food has come under attack due to their carcinogenic nature. Therefore the development and utilization of more effective antioxidants of natural origin, especially from the plants are required. Hence, the present study is focused on natural antioxidant compounds from fruit industry wastes *viz.* pomegranate, pineapple and orange peels.

The fruit wastes (pomegranate, pineapple and orange peel) were collected from local market at Tuticorin city. The peels were dried in hot air oven and ground into fine powder. The pomegranate, orange and pineapple peel powder were extracted using methanol, and the extracted solvents were pooled in the rotary

evaporator. Further was concentrated by removing the methanol under low pressure and temperature. The extraction yield was calculated by total extractable matter from the dry powder of pomegranate and orange peel. The highest yield was obtained from dried pomegranate peel powder i.e. 23.74% and orange peel powder, gave 20.06% where as in case of pineapple peel powder the was 17.11%. Combined fruit peel extracts prepared by mixing them in equal ratio or amount (10 gm each fruit peel extracts). After extraction of natural antioxidants, the total phenolic and flavonoid contents were quantified. The Total phenolic content of the PPE was higher (226 mg/g GAE) than CFPE (152 mg/g GAE), OPE (141.66 mg/g GAE) and PAPE (137 mg/g GAE). The total flavonoid content was just opposite to the total phenolic content. A higher value of 189.33 mg/g QE in PAPE as compared to PPE i.e.177.33 mg/g QE, CFPE 111.33 mg/g QE and OPE 88.66 mg/g QE was obtained.

The antioxidant activities of pomegranate, orange and pineapple and the combined one were measured by the DPPH. The DPPH radical scavenging activity of PPE extracts showed radical scavenging activity at the concentration of 4 µg/mL with the inhibitory activity of 88.46% at the same concentration BHA was almost equivalent to 91.87% while CFPE, PAPE and BHT showed at a concentration of 6 µg/mL i.e. 83.09%, 76.85%, 89.54% respectively. The OPE at 8 µg/mL i.e. 81.53%. Among all the extracts, PPE showed the higher radical scavenging activity followed by CFPE, OPE and PAPE.

Standardization of effective dose application of fruit peel extracts on sardine based on biochemical, microbiological and sensory parameter. Sardine was treated with 1% and 3% concentrations of fruits peel extracts and their combination, BHA and BHT (2 ppm) used as a reference. Treated and control samples were packed in LDPE pouch and kept in chilled storage (4±1˚C) for 72 hours and were assessed everyday by microbiological parameter-Total Plate Count, biochemical parameters like - TVB-N, TBA, PV, sensory evaluation by- Appearance (9 -point hedonic scale) and the Texture by TA-XT plus Texture Analyzer. The results showed that pomegranates peel extracts treated fish gave better results followed by CFPE, OPE and PAPE. Between BHA and BHT treated fish, BHA given better storage stability than BHT.

After standardization, the results showed that pomegranate peel extracts treated fish and BHA treated fish gave better storage stability. Hence, pomegranate peel extract was selected along with BHA for further study. As per standardization, raw materials was prepared, treated, packed and stored in chilled storage (4±1 ˚C) for 15 days under chilled storage condition and were assessed in every three days interval by microbiological, biochemical and sensory parameters.

The primary oxidation product PV was increased till 12^{th} for treated sample and till 9^{th} day for control after that decreased with increase in storage time during chilled storage conditions. The secondary oxidation product TBA was increased with increase in storage time. The highest increase of primary and secondary oxidation products was recorded in case of control sample and the lowest in case of TP3, followed by TB and TP1. The sample treated with TP3

extract was most effective for inhibiting the primary and secondary lipid oxidation parameters under the storage conditions.

The entire sample groups showed increase in nitrogenous compound during the storage study. The sample treated with PPE and stored at chilled condition was acceptable up to 12th day compared to control found acceptable for 6th day only.

The total plate count was increased with increase in storage time during chilled storage conditions. The highest increase of TPC was recorded in case of control sample and lowest in case of TP3, followed by TB and TP1. The sample treated with TP3 was most effective for inhibiting the TPC at all the storage conditions.

Based on the results of sensory score, the sample treated with TP1 and TP3 was appeared to be the best sensory score as compared to TB treated sample. The TP1, TP3 and TB sensory score was acceptable up to the 15th day of as compared to control which remained acceptable up to the 9th day. The texture profile analysis of sardine treated with TP1, TP3 and TB was assessed and based on the results the texture profile decreased with increase in storage based. The highest decrease of texture parameters were recorded in case of control and lowest in TP3, followed by TB and TP1.

In the present investigation it can be concluded that among the different and combined fruit peel extracts, pomegranate peel extract at 3% concentration (TP3) gave better results in delaying the lipid oxidation in sardine muscles. Hence, it was selected for further chilled storage studies. The present findings will be useful in leading to further experiments on the identification and characterization of natural

antioxidants sources that are responsible for preservation of eating quality and extended shelf life of fish and fishery products. Pomegranate peel extracts and combined peel extracts can be recommended for commercial application. The PPE and CFPE 30 gm each required for preserving 1 kg of fish to prevent oxidative rancidity.

VII. REFERENCES

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APPENDIX

TAMIL NADU Dr. J. JAYALALITHAA FISHERIES UNIVERSITY, NAGAPATTINAM
COLLEGE OF FISHERIES AND RESEARCH INSTITUTE
DEPARTMENT OF FISH PROCESSING TECHNOLOGY

Instruction: Please examine the different sample of Sardine fish and give the scores to them according to sensory scheme for sardine fish

Sampled after……days of storage

Sl. No.	Sensory Attributes	A	B	C	D
1	Appearance				
2	Colour				
3	Odour				
4	Texture				
5	Overall acceptability				
6	Remarks				

Hedonic scale:

Like extremely	:	9
Like very much	:	8
Like moderately	:	7
Like slightly	:	6
Neither like nor dislike	:	5
Dislike slightly	:	4
Dislike moderately	:	3
Dislike very much	:	2
Dislike extremely	:	1

Name:

Signature of Evaluator:

Date: