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Effect of Rosemary and Oregano Extracts Incorporated Chitosan Films on the Quality and Shelf Life of Indian Mackerel (*Rastrelliger kanagurta*) Steaks during Ice Storage

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A B S T R A C T

The effect of rosemary extract and oregano extract was compared with Butylated Hydroxytoluene (BHT) which was incorporated in chitosan film and studied the quality and shelflife of Indian Mackerel (*Rastrelliger kanagurta*) steaks during ice storage. The quality of the product was analysed by using biochemical methods (peroxide value, free fatty acid, thiobarbituric acid, trimethyl amino nitrogen, total volatile basic nitrogen, pH), microbial methods (total plate count) and sensory quality. The antioxidant properties of rosemary and oregano extracts were tested *in vitro* at varied concentrations (100 to 500 ppm) and growth inhibition was seen against gram positive and gram negative bacteria by disc diffusion method. It was observed that 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of Rosemary and Oregano extracts at 100ppm concentration were 77.37% and 62.86% respectively. Rosemary extract showed highest ferric reducing activity at all concentrations(100-500ppm) and exhibited highest reducing power at 500 µg /mL, almost equivalent to BHT at 200 mg/L. Rosemary extract exhibited more chelating activity compared to oregano extract, although both extracts were less efficient compared to synthetic metal chelator, Ethylene diamine tetraacetic acid (EDTA). Rosemary and Oregano extracts were potentially active against gram+ve bacteria whereas, it showed smaller zones of inhibition against gram-ve bacteria. The effect of 1% chitosan, 1% chitosan with 200ppm of BHT, 1% chitosan with 500ppm of rosemary and 1% chitosan with 500 ppm of oregano treatments on quality changes of Indian mackerel steaks during ice storage for 21 days were investigated.

Introduction

Indian mackerel (*Rastrelliger kanagurta*) a pelagic species belonging to the family Scombridae is found naturally and very abundantly in the east and west coast of India. It is commercially important fishery due to its food value and industrial use. Indian mackerel contributes about 9 % and forms the mainstay pelagic fishery after oil sardine. The consumption of Indian mackerel is either locally as fresh fish, iced or as frozen

products. Commercial use of Indian mackerel has been limited by the susceptibility of the fish to oxidative reaction of its lipids. Apart from lipid oxidation, the quality loss of the Indian mackerel was due to microbial spoilage, which is prime contributor for its spoilage. Oxidation can also cause other detrimental effects such as discoloration, vitamin destruction and decomposition of essential fatty acids, leading to organoleptic failure and a decrease in nutritive value (Sherwin, 1978). To retard such a quality loss, synthetic antioxidants and antimicrobials have been used to decrease lipid oxidation and microbial spoilage during the processing and storage of fish and fishery products (Boyd *et al.,* 1993).

Therefore, enhancing shelf life of seafood with natural preservatives and edible film is an important issue to eliminate economic losses and provide safe and good quality food to consumer and reach to distant markets (Kykkidou *et al.,* 2009). Edible films and coatings are used in a variety of applications in the food industry. The use of edible coating has a beneficial effect on the preservation of sea food products, since they act as barrier against moisture and oxygen penetration (Pereira *et al.,* 2010).

Chitosan and chitosan based materials can be used as edible films and coating. Chitosan is produced commercially by deacetylation of chitin (Mathur and Narang, 1990). It is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan, a.cationic polysaccharide mainly made from crustacean shells, is a well-known film forming biopolymer with strong antimicrobial & antifungal activities (Aider, 2010; Duan *et al.,* 2010). The antimicrobial activity of chitosan film is due to positively charged chitosan molecule act on negatively charged microbial cell membrane. The antioxidant activity of chitosan is to inhibit the reactive oxygen species present in lipid oxidation of food and biological systems. Chitosan can scavenge free radicals or chelate metal ions from the donation of hydrogen or the alone pairs of electron (Xie, 2001; Liu *et al.,* 2009; Onsosyen and Skaugrud, 1990). The current increase in consumer demand for synthetic antioxidants replace by the use of natural antioxidants and antimicrobial compounds has forced companies and researchers to explore different ways to improve their market penetration by offering products with improvements in quality, freshness and food safety. One of the more fashionable trends consists of the development of innovative biopolymers obtained from agricultural commodities and/ or food-waste products.

Plant extract of Rosemary (*Rosemarinus officinalis*) is one of the most effective spices widely used in food processing. It is the most important spices commercially available for use as an antioxidant and antimicrobial substance. The first use of an extract of rosemary leaves as an antioxidant was reported by Rac and Ostric (1955). The application of rosemary extracts in food had given a variety of results and these depend on the test model being used. Rosemary was considered as a lipid antioxidant, metal chelator and found to scavenge superoxide radicals. The capability of rosemary extracts in retarding lipid oxidation of different fish oils was reported by Bhale *et al.,* (2007). Oregano (*Origanum vulgare*) was very often used as a spices and its flavour is very popular with consumers all over the world. Oregano phenolics have significant antioxidant activity and are effective in the inhibition of all phases of the peroxidative processes by neutralizing free radicals, blocking the oxidation catalysis by iron and interrupting the lipid radical chain reactions (Dornan *et al.,* 2003). Primarily rosmarinic acid is the major phenolic

component of oregano extract, which can prevent colour deterioration (Hernandez *et al.*, 2009). The dried oregano has demonstrated *in vitro* antibacterial activity against a wide range of gram+ve and gram-ve microorganisms.

Materials and Methods

Materials

Preparation of chitosan film

The chitosan film was prepared by the casting method (Kanatt *et al.,* 2012). The known concentration of chitosan powder was taken and dissolved in 100 ml of 1% acetic acid solution. The chitosan and acetic acid solution were stirred continuously for 30 min's with the help of magnetic stirrer and 1 mL glycerol (film forming solution) was added as the plasticizer in the solution and again stirred for 15 min's. After filtration, known volume (25-30 ml each plate) of the solution was poured into the petri plates. These petri plates were dried at 65-70°C in hot air oven. After drying, immediately they were cooled to room temperature. Then 5 ml of 1M NaOH (sodium hydroxide) solution was added on the surface of dried film as it helps for easy peeling of film. Once the films were peeled, they were washed thoroughly in water, dried and used for further studies.

Standardization of chitosan for dip treatment

Preliminary experiments were conducted to standardize the various levels of Chitosan required for the preparation of the film and incorporated with mackerel steaks and to optimize processing conditions. Different concentrations about 0.25%, 0.5%, 0.75% and 1.0% of chiotsan film were prepared and to analyze the size and thickness. Among these concentration 1% level is give better thickness and size compared to the 2% (it give more thickness). Based on the analysis in order to find out a right standard level for the preparation of chitosan film selected and were used for storage studies.

Comparison between the chitosan and control

Before going to conduct the dip treatment in preliminary test between the chitosan treated sample and control sample. Both the samples were analyzing quality parameters including the biochemical, microbial and sensory characteristics shown in Table 1 and 2.

Based on the quality parameter analysis chitosan treated sample showed better quality compared to the control groups. So that the chitosan treated sample were kept as the control for present study.

Dip treatment

Indian Mackerel steaks were randomly assigned into four groups. Among these the first group steaks were coated with chitosan only (control). The second, third and fourth groups were treated with chitosan solution incorporated with 200ppm of BHT, 500ppm of Rosemary and 500ppm of Oregano respectively. The time for the dip treatment process for all the treatments is 10 min's.

Sampling

During ice storage studies of mackerel steaks samples were drawn randomly at an interval of every 3 days, up to 21 days in order to evaluate the lipid oxidation, microbiological, biochemical parameters and sensory parameter.

Plant varieties

Two varieties of plants *viz.*, Rosemary and Oregano were used for the study.

Glassware and packing material

All glassware's were procured from Merck, Borosil, Qualigen laboratories, India. 500g capacity High Density Polyethylene (HDPE) pouches (400 gauge) of size 24 x 17.8 cm were used for packaging of mackerel steaks.

Chemical and microbiological media

All the chemicals and reagents used in the present study were obtained from Merck (Mumbai), SD-fine chemicals (Mumbai) and Loba (Mumbai) were of analytical grade (AR) or guaranteed grade (GR). The glassware manufactured by Borosil, Technico, and Schott Duran was used during the study. The media used for microbiological studies were manufactured by Hi-Media (Mumbai). Chitosan powder was purchased from Nano Wings Pvt. Ltd., Khammam.

Bacterial cultures

Bacterial cultures, namely *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2688), *Bacillus subtilis* (NCIM 2063), *Salmonella typhium* (NCIM 2501) *and Pseudomonas fluorescens* (NCIM 2099) were brought from the Department of Fish Processing Technology, College of Fisheries, Mangalore, India. The above cultures were grown in nutrient agar media (Hi Media, Mumbai, India) at 37°C. Each bacterial strain was transferred from slants stored at 4-5°C to 10 ml nutrient broth and cultivated at 37°C for 24 h. Pre culture was prepared by transferring 1ml of this culture to 9ml nutrient broth and cultivated for 48 h.

Methods

Antioxidant capacity (AOC) of Rosemary and Oregano

The DPPH radical scavenging activity of rosemary and oregano at various concentrations was determined according to the method as described by Yen and Wu (1999). The ferric reducing antioxidant power of rosemary and oregano was measured to reduce ferric ions to ferrous ions as determined at different concentrations by the method of Oyaizu (1986). The chelating activity of rosemary and oregano at different concentration was measured by the method of Boyer and Mccleary (1987) and was compared with standard metal chelator EDTA at 1mM.

Antimicrobial activity of Rosemary and Oregano by disc diffusion method

The antibacterial test for rosemary and oregano were performed by the agar disc diffusion method (Bauer *et al.*, 1966; Nair and Chanda, 2005).

Chemical analysis

Peroxide value was determined according to Jacobs (1958). TBA value was determined as described by Tarladgis *et al.*, (1960), Color developed was measured using a UV-VIS spectrophotometer (M/s. UNICO spectrophotometer, USA) at 538 nm and expressed as mg malonaldehyde (MA) per kg of sample. Total Volatile Base Nitrogen (TVB-N) and Trimethyl amine Nitrogen TMA-N was determined by the method of Conway (1962) and expressed as mg/100 g of sample. pH value was determined according to APHA(1998) using a digital pH meter (M/s. Oakton, Eutech instruments, Malaysia) after homogenizing 5g of the fish sample with the 50ml of distilled water. Free fatty acid was (FFA) content in the lipid extract was determined by Olley and Lovern (1960) method.

Bacteriological analysis

All the microbial analysis was enumerated as per the procedures described in APHA (1992). The microbial count was estimated by spread

plate technique. 25 g of the sample was weighed aseptically and diluted with 225 ml of physiological saline solution. Samples were homogenized using stomacher (M/s. Lab-Med, England) and prepared serial dilutions at all possible aseptic precautions. Using the sterile pipette, 1ml of the supernatant was aseptically transferred into 9 ml of saline tube and mixed well using vortex mixer. Similarly, further decimal dilutions were prepared using physiological saline (0.85% sodium chloride solution).

Sensory analysis

Sensory characteristics of the fish steaks were evaluated by selected panel members who have experience in evaluation of similar products, on a ten-point scale (Indian Standard, 1971; Vijayan, 1984). Scores were assigned to '1' being the least and '10' being the highest for attributes as described by Vijayan (1984). The characteristics covered under the taste panel were appearance, color, flavor, taste, texture and overall acceptability for chitosan coating mackerel steaks treated with Rosemary and Oregano. Score 10-excellent to1-very dislike respectively for each of the sensory characteristics.

Statistical analysis

The Statistical Package for Social Sciences [SPSS 20 and IBM 2010] was used for analysis of the experimental results. The results were expressed as mean ± Standard Deviation (SD). Sufficient number of samples was carried out for each analysis.

Results and Discussion

Antioxidant activity of rosemary and oregano

The antioxidant potential of plant products and pure compounds was evaluated using numerous assays. The first step in these examinations is the screening of the potential activity by different in vitro tests. Each of those is based on one feature of the antioxidant activity, such as the ability of scavenging free radicals, the ferric reducing power assay, the chelating of metal ions. However, in order to get relevant data, a single method for testing antioxidant activities of plant products is not recommended due to their complex composition (Nuutila *et al.,* 2003). Therefore, the antioxidant activity of the tested rosemary and oregano has been evaluated in a series of in vitro tests.

DPPH radical scavenging activity rosemary and oregano

The DPPH radical scavenging activity of rosemary and oregano were shown in Table.3. The radical scavenging activity of the both the extracts were seen at different concentrations and with the increase in concentration, the radical scavenging activities of both the extracts decreased. At the same concentration used, the descending orders of DPPH radical scavenging activity of the tested compounds was as follows: Rosemary > Oregano.

The present results agreed with the findings of Hendel *et al.,* (2016) who reported that rosemary exhibited a high radical scavenging activity (11.741±0.004µg/ml) close to those of the tested synthetic antioxidants *viz.,* Ascorbic acid (3.036±0.217µg/ml), BHA (7.492±0.057µg/ml) and BHT (21.211±2.593µg/ml). Lugemwa *et al.,* (2013) also reported DPPH radical scavenging activities of several herbs and they found that oregano and rosemary showing LC 50 value of 592.5 and 414.2 mg of phenol/L respectively. The results of the present study can be compared with the findings of Khanum *et al.,* (2011) where they found that oregano exhibited maximum radical scavenging activity of 88.2% and 82.3% for aqueous and ethanolic extracts at 50 ppm concentration respectively.

Ferric reducing antioxidant power assay rosemary and oregano

In present investigation of rosemary and oregano were assayed for their ferric reducing activity at different concentration (100-500µg/mL) and the results are depicted in Table.3. The activity was compared with reference standard BHT at a concentration of 200mg/L. The reducing power of both the compounds increased with the increase in concentration ($p<0.05$). At the same concentration used, the descending order of FRAP of the compounds were as follows: Rosemary > Oregano.

The synthetic antioxidant BHTs showed maximum absorbance of 1.283 Abs at 200mg/L whereas Rosemary and Oregano showed higher ferric reducing capability of 2.162 and 1.379Abs respectively at 500mg/mL. The findings were agreed with Fernandes *et al.,* (2016) who reported that rosemary and oregano showed ferric reducing ability of 361.57±33.72 and 472.32±15.96 respectively. These findings are not in agreement with those reported by Shan *et al.,* (2005) who noticed oregano extracts (1.01 mmol trolox/g dw) showed higher ferric reducing antioxidant power compared with that of rosemary extracts (0.38 mmol trolox/g dw).

Metal chelating activity of rosemary and oregano

In present investigation of rosemary and oregano were assayed for their metal chelating activity at different concentration and the results were depicted in Table.3. The activity was compared with synthetic metal chelator (EDTA) at 1.0mM. The maximum metal chelating activity of rosemary and oregano were seen at 500mg/L which was 50.28% and 39.16% whereas EDTA at 1.0 mM showed 85.65%. The metal chelating ability of both the compounds was very less at lower concentrations but increased with increase in concentration. The metal chelation activity of rosemary extract were checked by El-Beltagi and Badawi (2013) and they reported that the percentages of metal scavenging capacity at 200 µg/ ml of tested methanol extracts of rosemary and EDTA was found to be 38.31 and 51.21% respectively. Bejaoui *et al.,* (2013) studied a substantial metal chelating capacity of methnolic extract, ethanolic extract and water extract and documented metal chelating activity of 76.98, 48.95 and 31.68% respectively.

In vitro antimicrobial activity of Rosemary and Oregano

The antimicrobial activity of rosemary and oregano were checked at 5 mg/ ml and the results are shown in Table 4. Among the two extracts tested against gram+ve and gram–ve bacteria, rosemary showed higher antimicrobial activity compared with oregano. The present results of the study can be compared with the findings of Zhang *et al.,* (2016) who had investigated antimicrobial activity of rosemary at 5, 10, 20, 40 mg/ml concentration against *E.coli* and *Pseudomonas fluorescens*.

The zone of inhibition was found to be 12.13, 13.84, 16.81, 17.54 for *E.coli* and 9.40, 11.45, 13.05 and 17.73 for *Pseudomonas fluorescens* at 5, 10, 20, 40 mg/ml concentration respectively. Seydim and Sarikus (2007) reported that oregano were tested against *E.coli, Staphylococcus* and *Salmonella enteritidis* and the zone of inhibition were found to be 777.72, 957.25 and 883.34 mm^2 respectively at 4% concentration. The higher antimicrobial activity of rosemary and oregano may be presence of core compounds like Thymol and Carvacrol which might play an important role in their antimicrobial activity.

Proximate composition of Indian mackerel

In present study the proximate composition of Indian mackerel had moisture content of 74.48%, protein content of 17.02%, Fat content of 6.52% and Ash content of 1.30%. Among this composition moisture content was very high compared to protein, fat, ash. The present study results were compared to the Sofi *et al.,* (2015), who documented that proximate composition of Indian mackerel were shown 71.02% of moisture, 21.02% of protein, 6.09% of fat and 1.20% of ash respectively. The results of this study were in agreement with the findings of Lakshmisha *et al.,* (2014) for moisture and lipid content ranged between 71.31 to 76.63% and 5.90 to 7.25% respectively.

Chemical analysis

Changes in peroxide value

In the present investigation, peroxide value of all the treatments increases throughout the storage period showed Table.5. In this study, PV value initially in all treatment groups were similar and increased during the increasing of storage period. Chitosan treated sample showed significantly ($p<0.05$) higher PV value compared to the BHT, rosemary and oregano.

The increase in PV in all the samples indicated that, the samples were in propagation stage of lipid oxidation with a lower rate of decomposition of hydroperoxides. The increase in peroxide value of Indian mackerel during ice storage was also reported by Sofi *et al.,* (2015). Active packaging with chitosan film will help in reduction of hydroperoxide formation as reported by Coban and Pelin Can, (2013) and they found that primary lipid oxidation can be minimized by active packaging film containing rosemary extract in smoked rainbow trout. The inhibition of peroxides was concentration-dependent which showed a direct relationship between the polyphenolic concentration and the inhibitory efficiency as studied by Bensid *et al.,* (2014) who reported that beheaded anchovy treated with oregano lowers the rate of lipid oxidation by 1.5 times than that of untreated s amples. Other researchers also found that oregano was effective in controlling primary lipid oxidation as documented by Tsimidou *et al.,* (1995) who reported that 0.5% oregano having same effect as BHT at 200 ppm.

Changes in Thiobarbituric acid (TBA) during ice storage

The TBA value was used to measure the rancidity in fish and fishery products. Rancidity in fishery products was measured in terms of malonaldehyde content. In the present study, changes in TBA content of chitosan treated mackerel steaks during ice storage were represented in Table.6. The chitosan treated sample were showed significantly ($p<0.05$) higher TBA value compared to rosemary and oregano treated groups.

Li *et al.,* (2013) reported that chitosan film coating used directly on the surface of fish might act as barrier between fish meat and its surroundings, thus cutting down diffusion of oxygen to the fish meat surfaces. Bensid *et al.,* (2014) reported that TBA value decreases with the effect of oregano on gutted and beheaded anchovy.

The lowering in TBA value for control and oregano treated samples were found to be 8.77 and 4.81 mg malonaldehyde/kg of sample at the end of 12 days of storage. Ozogul *et al.,* (2010) observed the prevention of lipid oxidation using rosemary extract. They reported that the TBA formation in 1 and 2% rosemary treated sample were found to be 1.49 and 0.65 mg malonaldehyde/kg sample respectively at the end of 20 days of storage.

Table.1 Biochemical changes of control and chitosan treated samples

Storage period (Days)	Biochemical changes			
	Control		Chitosan	
	PV (meq O_2/kg of fat)	TBA(mg of MA/kg of sample)	PV (meq O_2/kg of fat)	TBA(mg of MA/kg of sample)
0	1.50±0.07^{b}	0.26±0.11^{b}	1.20±0.05^{a}	0.22±0.18^{a}
3	3.26±0.07^{b}	0.76±0.26^{b}	1.86±0.16^{a}	0.59±0.32^{a}
6	5.96±0.34^{b}	1.30±0.12^{b}	2.79±0.23^{a}	1.02±0.30^{a}
9	10.25±0.14^{b}	1.52±0.19^{b}	3.65±0.89^{a}	1.25±0.10^{a}
12	13.30±0.02^{b}	1.76±0.08^{b}	5.08±0.12^{a}	1.47±0.21^{a}
15	15.02±0.84^{b}	2.07±0.29^{b}	6.27±0.43^{a}	1.62±0.03^{a}

*Each value is represented by the mean ± SD of n=3
^{abcd} Indicate significant difference among treatments ($p<0.05$)

Table.2 Microbial and Sensory changes of control and chitosan treated samples

Storage period (Days)	TPC (cfu/gram of meat)		Sensory	
	Control	Chitosan	control	Chitosan
0	4.65±0.07^{b}	4.06±0.14^{a}	9.00±0.02^{a}	9.26±0.15^{b}
3	5.29±0.08^{b}	4.72±0.11^{a}	8.02±0.07^{a}	8.35±0.15^{b}
6	6.49±0.15^{b}	5.62±0.03^{a}	7.02±0.03^{a}	7.89±0.31^{b}
9	7.72±0.27^{b}	6.27±0.14^{a}	5.89±0.20^{a}	6.26±0.40^{b}
12	9.28±0.13^{b}	6.80±0.27^{a}	5.02±0.41^{a}	5.82±0.03^{b}
15	10.19±0.33^{b}	7.73±0.28^{a}	3.68±0.12^{a}	5.62±0.23^{b}

*Each value is represented by the mean ± SD of n=3
^{abcd} Indicate significant difference among treatments ($p<0.05$)

Table.3 Antioxidant activity of Rosemary and Oregano

Antioxidant activity	DPPH		FRAP		MCA	
	Rosemary	**Oregano**	**Rosemary**	**Oregano**	**Rosemary**	**Oregano**
100ppm	77.37±0.77^{d}	62.86±0.67^{e}	0.528±0.03^{a}	0.052±0.01^{a}	23.17±0.14^{a}	15.41±0.38^{a}
200ppm	76.54±0.35^{d}	60.38±0.57^{d}	0.861±0.07^{b}	0.426±0.08^{b}	31.28±0.78^{b}	22.96±0.29^{b}
300ppm	75.17±0.22^{c}	58.16±0.76^{c}	1.287±0.26^{c}	0.780±0.06$_{c}$	39.63±0.78^{c}	30.30±1.05^{c}
400ppm	73.18±0.72^{b}	56.03±0.38^{b}	1.849±0.06^{d}	0.990±0.01^{d}	44.40±1.83^{d}	34.37±0.97^{d}
500ppm	69.92±0.65^{a}	54.01±0.81^{a}	2.162±0.06^{e}	1.379±0.04^{e}	50.28±0.93^{e}	39.16±0.41^{e}
BHT (200 ppm)	82.69±0.46	82.69±0.46	1.282±0.06	1.282±0.06	-	-
EDTA(1.0mm)	-	-	-	-	85.65±0.45	85.65±0.45

DPPH- Diphenyl-1 picrylhydrazyl: FRAP-Ferric reducing power assay:
MCA-metal chelating activity
*Each value is represented by the mean ± SD of n=3
^{abcd} Indicate significant difference among treatments ($p<0.05$).

Table.5 Biochemical changes in Indian mackerel steaks with the effect of chitosan treated Rosemary and Oregano during chill storage

Parameter	Treatment	Storage days							
		0	3	6	9	12	15	18	21
PV(mg of hydro peroxide/kg of sample)	Chitosan	1.08±0.06^{b}	3.59±0.16^{c}	6.60±0.36^{d}	8.40±0.34^{d}	10.38±0.23^{d}	11.46±0.17^{d}	12.67±0.18^{d}	14.77±0.16^{d}
	Chitosan +BHT	0.90±0.06^{a}	2.47±0.11^{a}	4.16±0.14^{a}	6.21±0.16^{a}	8.13±0.10^{a}	9.19±0.14^{a}	10.22±0.14^{a}	11.80±0.12$_{a}$
	Chitosan +Rosemary	0.96±0.06^{a}	2.79±0.18^{b}	4.66±0.34^{b}	6.43±0.18^{b}	8.41±0.19^{b}	9.65±0.18^{b}	10.60±0.11^{b}	11.97±0.26^{ab}
	Chitosan + Oregano	0.96±0.06^{a}	2.86±0.14^{b}	4.86±0.13^{bc}	6.61±0.26^{c}	8.62±0.27^{c}	9.85±0.11^{c}	10.86±0.10^{bc}	12.46±0.35^{c}
TBA(mg MA/kg of sample)	Chitosan	0.37±0.020^{a}	0.64±0.015^{b}	0.84±0.025^{b}	1.29±0.005^{c}	1.70±0.005^{b}	2.22±0.010^{d}	2.56±0.000^{d}	2.81±0.000^{d}
	Chitosan +BHT	0.36±0.026^{a}	0.59±0.015^{ab}	0.73±0.020^{a}	0.96±0.011^{a}	1.24±0.010^{a}	1.57±0.005^{a}	1.74±0.025^{a}	2.05±0.005^{a}
	Chitosan +Rosemary	0.35±0.015^{a}	0.56±0.020^{a}	0.73±0.032^{a}	0.98±0.005^{a}	1.25±0.010^{a}	1.61±0.010^{b}	1.84±0.062^{b}	2.12±0.005^{b}
	Chitosan + Oregano	0.36±0.026^{a}	0.63±0.050^{b}	0.73±0.025^{a}	1.01±0.025^{b}	1.37±0.025^{b}	1.68±0.005^{c}	1.91±0.025^{c}	2.22±0.005^{c}
FFA(% of oleic acid)	Chitosan	1.19±0.34^{c}	2.59±0.12^{c}	4.52±0.14^{d}	5.26±0.16^{d}	6.85±0.28^{d}	7.58±0.11^{d}	8.74±0.18^{d}	10.24±0.10^{d}
	Chitosan +BHT	0.89±0.22^{b}	1.85±0.14^{a}	2.92±0.11^{a}	3.68±0.18^{a}	4.13±0.14^{a}	5.39±0.10^{a}	6.21±0.10^{a}	8.75±0.13^{a}
	Chitosan +Rosemary	0.89±0.22^{b}	2.14±0.12^{b}	3.21±0.20^{b}	4.65±0.15^{b}	5.41±0.25^{b}	6.24±0.20^{b}	7.21±0.12^{b}	9.23±0.10^{b}
	Chitosan + Oregano	0.67±0.22^{a}	2.29±0.11^{ab}	3.69±0.12^{c}	4.86±0.10^{c}	5.61±0.27^{b}	6.58±0.20^{bc}	7.55±0.11^{c}	9.60±0.15^{ab}
TMAN(mgN/100g of sample)	Chitosan	1.63±0.12^{b}	0.35±0.015^{a}	6.35±0.14^{c}	9.81±0.18^{c}	10.50±0.16^{c}	13.75±0.13^{c}	15.45±0.13^{d}	20.18±0.14^{d}
	Chitosan +BHT	1.43±0.06^{ab}	2.52±0.18^{a}	5.42±0.28^{a}	6.40±0.22^{a}	6.39±0.18^{a}	9.77±0.11^{a}	12.52±0.12^{a}	15.41±0.08^{a}
	Chitosan +Rosemary	1.62±0.21^{b}	3.51±0.18^{b}	5.68±0.12^{ab}	6.74±0.08^{ab}	8.81±0.13^{b}	10.32±0.11^{b}	14.31±0.54^{b}	16.82±0.17^{c}
	Chitosan + Oregano	1.63±0.22^{b}	3.39±0.06^{ab}	5.79±0.16^{ab}	6.80±0.17b	8.80±0.17^{b}	10.53±0.17^{b}	14.80±0.18^{c}	16.79±0.18^{c}
TVBN (mgN/100g of sample)	Chitosan	2.56±0.14^{c}	5.38±0.20^{c}	9.35±0.24^{c}	13.51±0.27^{c}	18.65±0.14^{c}	25.45±0.21^{d}	28.40±0.15^{d}	32.16±0.18^{d}
	Chitosan +BHT	1.60±0.20^{a}	3.57±0.14^{a}	7.37±0.15^{a}	10.29±0.15^{a}	16.57±0.14^{a}	18.43±0.18^{a}	20.13±0.10^{a}	25.47±0.15^{a}
	Chitosan +Rosemary	1.67±0.13^{a}	4.37±0.15^{b}	8.39±0.16^{b}	11.22±0.18^{b}	17.610.10^{b}	20.46±0.17^{b}	24.21±0.12^{b}	26.34±0.11^{b}
	Chitosan + Oregano	1.76±0.12^{ab}	4.40±0.23^{b}	8.21±0.13^{b}	11.54±0.23^{b}	17.59±0.11^{b}	21.59±0.25^{c}	27.55±0.10^{c}	28.57±0.14^{b}
pH	Chitosan	6.37±0.04^{c}	6.52±0.09^{c}	6.63±0.16^{c}	6.67±0.13^{c}	6.82±0.15^{b}	6.79±0.16^{b}	6.92±0.32^{c}	7.23±0.18^{c}
	Chitosan +BHT	6.19±0.20^{a}	6.25±0.12^{a}	6.42±0.16^{a}	6.50±0.18^{a}	6.61±0.17^{a}	6.69±0.16^{a}	6.72±0.29^{a}	6.77±0.10^{a}
	Chitosan +Rosemary	6.28±0.19^{b}	6.48±0.12^{b}	1.57±0.17^{b}	1.59±0.18^{ab}	6.65±0.15^{a}	6.72±0.16^{ab}	6.74±0.22^{a}	6.80±0.22^{ab}
	Chitosan + Oregano	6.29±0.13^{b}	6.49±0.17^{b}	6.52±0.19^{b}	6.60±0.18^{bc}	6.69±0.17^{a}	6.74±0.15^{b}	6.81±0.17^{b}	6.93±0.26^{b}

*Each value is represented by the mean ± SD of n=3 and ^{abcd} Indicate significant difference among treatments ($p<0.05$)

Table.6 Microbial and sensory changes in Indian mackerel steaks with the effect of chitosan treated Rosemary and Oregano during chill storage

Parameter	Treatment	Storage Days							
		0	3	6	9	12	15	18	21
TPC	Chitosan	4.04±0.14^{a}	4.64±0.11^{a}	5.18±0.13^{a}	6.27±0.13^{a}	6.80±0.13^{b}	7.10±0.13^{b}	7.43±0.13^{b}	8.61±0.14^{c}
	Chitosan +BHT	4.43±0.10^{a}	4.86±0.11^{b}	5.35±0.20^{c}	6.40±0.15^{c}	7.02±0.11^{c}	7.21±0.24^{c}	7.89±0.11^{d}	8.88±0.10^{d}
	Chitosan +Rosemary	4.03±0.26^{a}	4.69±0.14^{a}	5.32±0.10^{c}	6.37±0.05^{b}	6.78±0.12^{a}	6.86±0.18^{a}	7.12±0.16^{a}	8.39±0.20^{a}
	Chitosan + Oregano	4.19±0.15^{a}	4.61±0.26^{a}	5.27±0.08^{b}	6.35±0.18^{b}	6.82±0.30^{a} b	6.97±0.08^{a} b	7.55±0.08^{b} c	8.55±0.10^{b}
Over all acceptability	Chitosan	9.26±0.15^{a}	8.16±0.15^{a}	7.16±0.15^{a}	6.38±0.17^{b}	6.26±0.11^{a}	5.46±0.20^{a}	4.33±0.15^{b}	3.33±0.15^{b}
	Chitosan +BHT	9.50±0.10^{a}	8.41±0.18^{a}	7.41±0.20^{a}	7.13±0.11^{a}	6.60±0.26^{a}	5.60±0.26^{a}	5.32±0.17^{a}	4.81±0.14^{a}
	Chitosan +Rosemary	9.40±0.20^{a}	8.30±0.17^{a}	7.46±0.15^{a}	6.41±0.18^{b}	6.53±0.25^{a}	5.30±0.10^{a}	5.21±0.20^{a}	4.29±0.14^{a}
	Chitosan + Oregano	9.33±0.15^{a}	8.20±0.10^{a}	7.23±0.15^{a}	6.46±0.20^{b}	6.46±0.25^{a}	5.40±0.10^{a}	5.01±0.28^{a}	4.38±0.14^{a}

*Each value is represented by the mean ± SD of n=3

^{abcd} Indicate significant difference among treatments ($p<0.05$)

Table.4 Antimicrobial activity of rosemary and oregano

Microorganisms	Zone of inhibition in mm		
	Rosemary	**Oregano**	**Ampicillin**
S. aureus	19.56±1.23	15.37±0.59	38.16±0.83
B.subtilis	18.49±0.34	15.69±0.28	32.56±1.56
P.fluorescens	11.03±0.89	9.05±0.14	30.75±0.12
S.typium	20.72±0.43	11.09±0.71	33.26±0.51
E.coli	10.37±0.03	12.03±0.37	24.08±0.86

Changes in Free Fatty Acid (FFA) content during ice storage

In the present investigation, free fatty acid of all the treatments increases throughout the storage period was shown Table.5. FFA value was initially similar in all treatment groups during increase storage period chitosan treated sample shown significantly ($p<0.05$) higher than oregano, rosemary and BHT. The present Ozogul *et al.*, (2010) observed the effect of prevention of lipid hydrolysis by using rosemary extract and the results were in agreement with the present study that FFA formation of 1% rosemary treated sample were found to be 5.98 and 2% rosemary treated sample were found to be 6.13% of oleic acid/ kg of fat respectively at the end of 20 days of ice storage. Bensid *et al.*, (2014) reported that the oregano treated gutted and beheaded anchovy during chill storage. They stated that the FFA formation of oregano treated sample was found to be 5.39% of oleic acid/kg of fat respectively at 12 days ice condition whereas in control sample free fatty formation was 5.98% of oleic acid/kg of fat respectively. These results might be attributed to the effect of phenolic compounds in plant extracts, which lowers liberation of free fatty acids due to the inhibition of enzymatic action. Reesha *et al.*, (2015) studied that chitosan / low density polyethylene (LDPE) composite films in tilapia during chill storage. They reported that the FFA formation was found to be 11.00% of oleic acid/kg of fat in 1% chitosan treated samples at 30 days chill storage. It is concluded that rosemary and oregano extracts with chitosan coating helps in lowering the lipid hydrolysis of Indian mackerel steaks during ice storage.

Changes in Trimethylamine Nitrogen (TMA-N) content during ice storage

Trimethylamine nitrogen (TMA- N) level in fish is an important factor in the subjective evaluation of fish quality because of its close association with fish spoilage (Chang and Creaser, 1976; Love, 1992). In the present study TMA-N values were shown increasing trend throughout the storage period shown Table: 6. The present results can be compared with the findings of Mohan *et al.*, (2012) who studied the effect of chitosan edible coating on double filleted Indian oil sardine during chilled storage. They reported that initially TMA-N content for 1% chitosan treated sample was found to be 6.01mg N/100g and reaches maximum of 19.92 mg N/100g whereas 2% chitosan treated sample showed maximum of 17.19 mg N/100g in respectively after 30 days of storage period. Ozyurt *et al.*, (2012) studied the effect of rosemary extract on the oxidative stability and biogenic amine formation in sardine during chilled storage. They reported that the TMA-N values were recorded as 22.23 and 18.43mg N/100g for 0.05 and 0.1% rosemary after 25 days of chilled storage. It can be concluded that treatment of rosemary and oregano extract significantly affected the accumulation of TMA-N content in fish muscle.

Changes in Total Volatile Base-Nitrogen (TVB-N) content during ice storage

In the present investigation, TVB-N content of mackerel steaks were shown (Table:5) increase in TVB-N levels in fish may result from deamination of free amino acids, oxidation of amines and degradation of nucleotides by autolytic enzymes and microbial activity (Ocano-Higuera *et al.,* 2011). Li *et al.,* (2013) studied the quality changes of refrigerated red drum fillets using chitosan coating with natural preservative and reported that TVB-N content reaches maximum of 38.17 and 33.69 mg-N/100g after 20 days of ice storage for grape seed and tea polyphenols respectively, whereas untreated samples showed TVB-N content of 51.25 mg-N/ 100g at the end of 20 days of ice storage. Polyphenols from natural sources having antimicrobial activity which helps in reduction of bacterial flora in fish and fishery products as reported by Ozogul *et al.,* (2010) who reported that TVB-N content was found to be 34.29 mg-N/100 g at day 13 for untreated samples whereas 33.64 and 35.82 mg-N/100 g at day 17 for 1 and 2% rosemary extract respectively. The reduction in TVB-N content might be due to antimicrobial activity of rosemary extracts. The present results of the investigation can be compared with the findings of Bensid *et al.,* (2014) who reported that TVB-N content of oregano treated gutted and beheaded anchovy fillets during ice storage was shown maximum of 38.10 mg-N/100g of sample whereas, untreated samples showed maximum of 60.23 mg- N/100g of sample after 12 days ice condition.

Changes in pH during ice storage

An important determination of fish quality texture measurement and gaping in fish fillets which were influenced by pH (Love, 1992). The increase of pH values during the storage period may be attributed to accumulation of alkaline compounds, such as ammonia, which mainly derived from microbial action during fish muscle spoilage. Remya *et al.,* (2016) studied the chitosan based active films used in barracuda packing during chill storage. They reported that the pH value of chitosan with ginger essential oil was shown 6.9 whereas, untreated group showed 7.3 at the end of storage. Ozyurt *et al.,* (2012) studied that rosemary extract on the oxidative stability and biogenic amine formation in sardine during chilled storage. They documented that maximum pH values attained by untreated sample was 7.23 whereas 0.05% and 0.1% rosemary extract showed pH values of 7.26 and 6.9 respectively. The present results of oregano treated samples can be correlated with Bensid *et al.,* (2014) who observed that there is steady rise in pH during the ice storage period. The oregano treated samples showed maximum pH of 6.93 whereas untreated sample showed 7.3 at the end of 12 day storage.

Changes in Total Plate Count of mackerel steaks during ice storage

In the present study, changes in TPC content of chitosan treated mackerel steaks with rosemary and oregano during ice storage are represented in Table.7. The present findings can be correlated with the findings of Ozogul *et al.,* (2010) who observed prevention of microbial spoilage in oil sardine by using rosemary extract during 20 days of ice storage. They reported that untreated samples were in acceptable condition up to 10 days whereas 1% & 2% rosemary treated samples where in acceptable condition up to 13 and 17 days respectively. Mohan *et al.,* (2012) studied the chitosan edible coating of double filleted Indian oil sardine during 11 days of ice storage. They found that untreated samples were found in acceptable condition up to 9^{th} day whereas 1% and 2% chitosan treated samples were in acceptable condition

up to 11 days of ice storage. According to the (ICMSF, 1986) total aerobic plate count limit for fresh fish is 10^7 cfu/g (7 cfu/g). Based on ICMSF, (1986) the results of the present study crossed the limit on 18th day of ice storage for rosemary and oregano treated samples whereas untreated samples reached maximum on 12th day of ice storage.

Changes in organoleptic characteristics of mackerel streaks during ice storage

The overall acceptability score is the mean of attributes such as color, texture, appearance, flavor and taste. In the present study, overall acceptable scores were depicted in Table.6. The chitosan treated sample shown sensory rejection on 15th day ice storage. Decreasing in sensory score indicated the loss of freshness in samples; this could be due to lipid oxidation and microbial spoilage (Connell, 1995). Cai *et al.,* (2014) studied the quality changes of chitosan coated with ergothioneine in Japanese sea bass. They reported that the chitosan treated sample did not exhibit these characteristics even on the 16th day of storage whereas the uncoated samples were shown the limit of acceptability on 8th day. Bensid *et al.,* (2014) studied the oregano treated gutted and beheaded anchovy during ice storage. They reported that the sensory scores in both control and treated groups declined throughout the 12 days of ice storage.

The DPPH radical scavenging activity of both the compounds showed descending manner, rosemary showed more ferric reducing power than oregano and metal chelating activity of the both compounds was showed less chelation than compared the synthetic compounds. The gram -ve bacteria showed more inhibition zone compared to the gram +ve bacteria in both the compounds. The results indicated that both rosemary and Oregano extracts exhibited good antioxidant and antimicrobial properties by extending the shelf life of Indian mackerel steaks up to 3 days. Among the rosemary extract and oregano extracts, rosemary extract exhibited higher antioxidant and antimicrobial properties than oregano extract.

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