Indian J. Fish., 67(1): 114-122, 2020
DOI: 10.21077/ijf.2019.67.1.91361-15

Chitosan and lemon peel extract coating on quality and shelf life of yellowfin tuna (*Thunnus albacares)* meat stored under refrigerated condition

S. SABU*, T. ASHITA AND S. STEPHY
School of Industrial Fisheries, Cochin University of Science and Technology, Lake side campus, Kochi - 682 016 Kerala, India
e-mail: sabuif@gmail.com

ABSTRACT

The present study investigated the effect of chitosan combined with lemon peel extract coating on the quality and shelf life of refrigerated yellowfin tuna meat using physicochemical, microbial and sensory assessments. Fresh yellowfin tuna meat as chunks were divided into five lots and coated with lemon peel extract (LPE) and chitosan (CH) at different concentrations *viz.,* control, C (0%), LPE1%, CH1%, LPE+CH1% and LPE+CH2% (w/v). Sensory, biochemical and microbial quality of the samples were observed for 12 days during 4°C refrigerated storage. Sensory evaluation revealed that shelf life of yellowfin tuna under the study was 6 days for control, 8 days each for LPE (1%) and CH (1%), 10 and 12 days for LPE+CH (1%) and LPE+CH (2%) respectively. Significantly higher pH, total volatile basic nitrogen (TVB-N), tri-methyl amino nitrogen (TMA-N), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) values were recorded in control samples than coated samples ($p<0.05$). Significant reduction in microbial counts were recorded in CH+LPE treated samples ($p<0.05$) compared to the LPE or CH coating alone, in the later stages of storage. Coated samples with combination of LPE+CH 1% and 2% indicated better storage qualities compared to other treatments. The present study revealed that LPE along with chitosan edible coating enhanced the shelf life of yellowfin tuna meat.

Keywords: Antibacterial, Antioxidant, Chitosan, Lemon peel extract, Shelf life, Yellowfin tuna

Introduction

Yellowfin tuna (*Thunnus albacares)* occupies a prime position in the international seafood trade due to its good nutritional value; protein composition and textures. The major share of this species caught and marketed is in the freshest form (*Sashimi* grade) around the world (Jinadasa *et al.,* 2015). Bulk of the yellowfin tuna landed in harbours or fish landing centers in India are harvested by traditional fishermen and are considered as not eligible for *Sashimi* grade. Lack of specialised fishing vessels, preservation techniques for oceanic tuna resources, cold chain facilities as per the international standards are affecting the quality of the harvested tuna caught by the traditional fishermen of India. Harvested tuna from India are marketed as raw material for canneries and for direct consumption in fresh, chilled or frozen forms (Mohan *et al.,* 2015). There is a growing demand for chilled yellowfin tuna meat in ready-to-prepare form in the domestic markets of India (Jinadasa *et al.,* 2015). Artificial preservatives are proved to be effective in reducing spoilage and to ensure long shelf life (Yuan *et al*., 2016). Chemical preservatives such as butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) are used as antioxidants for food which could be toxic for consumers (Leclercq *et al.,* 2000; Maziero *et al*., 2001). Edible coating technology using natural antioxidants and antimicrobial agents have been intensively examined as safe alternatives to synthetic compounds (Shahidi, 2004; Encarnacion *et al*., 2012; Chaparro-Hernandez *et al.,* 2015; Yuan *et al*., 2016; Rao *et al.,* 2017).

Lemon (*Citrus limon)* belonging to the family Rutaceae (Dhanavade *et al*., 2011) is one of the major fruit produced in the world in terms of quantity (Miran *et al*., 2016). Lemon fruit contains unique compounds (flavanoes, flavanone, glycosides and polymethoxylated flavones) which possess antimicrobial and anti-inflammatory properties (Wanpeng *et al.,* 2017), which are rarely seen in other plants (Diankov *et al.,* 2011). Bulk of the fruit wastes including lemon peels are wasted in India (Pathak *et al*., 2017). Chitosan is a natural biopolymer which is non-toxic and exhibits antifungal and antibacterial activity (Kurita, 2006; Gomez-Estaca *et al.,* 2009). Chitosan (poly-b-(1–4)-D-glucosamine) and its derivatives, alone or in combination with natural or synthetic materials are good candidates for edible coating of foods (Kong *et al*., 2010; Ojagh *et al*., 2010a).

Chitosan coating in fruit (Lin and Zhao, 2007), seafood (Chaiyakosa *et al*., 2007) and meat products (Sagoo *et al*., 2002) has revealed the potential of this edible material in prolonging the storage life and controlling

food spoilage. Combinations of chitosan and herbal extracts and essential oils such as cinnamon oil (Ojagh *et al.,* 2010b), tea polyphenols and rosemary extract (Li *et al*., 2012) and pomegranate peel extract (Yuan *et al.,* 2016) have been tested previously for extending the shelf life of fresh fish samples. Chitosan in combination with grape seed extract and tea polyphenol extracts extended the quality of red drum fillets (Li *et al.,* 2013). Chitosan and citric acid as well as chitosan and licorice extract have been tested for preventing lipid oxidation and for inhibiting microbial growth in *Lateolabrax japonicus* (Qiu *et al*., 2014). Inhibition of microbial growth and pH value of tuna fillets coated with Chinese lemon extract and chitosan have also been studied (Renur *et al.,* 2016). The purpose of the present study was to evaluate the efficacy of chitosan coating combined with lemon peel extract (phenolics and flavonoids) on the quality and shelf life of yellowfin tuna meat stored under refrigerated condition (4±1°C) for 12 days.

Materials and methods

Raw materials and chemicals

Fresh yellowfin tuna was purchased from a local market in Thoppumpady, Ernakulam, Kerala, India. Fresh lemon (*Citrus limon)* fruits were obtained from Ernakulum market, Kerala, India. Food grade chitosan (88% degree of deacetylation having an average molecular weight of 30 kDa) obtained from M/s India Seafood's, Kochi, Kerala, India was used for the experiment. All other reagents and chemicals used in the study were of analytical grade and were procured from Merck (Mumbai, India).

Preparation of lemon peel extract

Lemon was manually peeled and the peels were then cleaned and dried overnight at 50°C in a hot air oven to reduce the moisture content. Dried peels (moisture content: 8.0±2.0%) were converted to powder and extract was prepared by following the procedure detailed by Singh and Immanuel (2014). Briefly, 10 g of the dried powder and 100 ml of ethanol in a conical flask plugged with cotton was kept in an orbital shaker at 120 rpm for 24 h at room temperature (RT). Extract was filtered using Whatman (No.1) filter paper and concentrated under vacuum at 40°C. The dry extract was stored at 4°C until further use.

Preparation of coating solutions

Chitosan (1 g) was dissolved in 1 ml acetic acid containing 100 ml of distilled water and stirred for 1 h at room temperature to obtain 1% chitosan solution (CH1%). Lemon peel extract (LPE1%) dipping solution was prepared by dissolving 1 ml LPE in 100 ml distilled water, while 50% of CH1% - 50% of LPE1% and 100% of CH1%+100% of LPE1% solutions were used as LPE+CH1% and LPE+CH2% respectively.

Coating procedure for fish samples

Fresh yellowfin tuna meat was brought to the laboratory under iced condition. After washing and cleaning, the meat was made into chunks and randomly assigned into five groups *viz*., control C (uncoated), LPE1%, CH1%, LPE+CH1% and LPE+CH2% groups after washing in chilled distilled water. The meat samples were dipped in the prepared solutions at a fish:solution ratio of 1:2 (w/v) at 4°C for 20 min. Control meat was dipped in chilled distilled water for 20 min. After dipping, the chunks were drained at ambient temperature for 3 min. The coated and uncoated samples were then packed in sterile polyethylene bags and stored under refrigerated condition (4±1°C). Samples representing all regions of the chunks of the respective lots (in correct quantities) were weighed and transferred for biochemical and microbiological analysis at every 2 day interval.

Determination of total phenolic, total flavonoid contents and antioxidant capacity of LPE

Folin-Ciocalteu's reagent method (McCune and Johns, 2002) was followed for determining the total phenolic content of LPE. Total phenolic content was expressed in terms of gallic acid equivalent for lemon peel (mg of gallic acid per gram (mg GAE g^{-1}) of extracted compound). The flavonoid content of LPE was determined using the aluminium chloride colourimetric method (Chang *et al.,* 2002). The result was expressed in terms of mg Quercetin equivalent per gram (mg QE g^{-1}) of extracted compound. DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity was determined following Singh and Immanuel (2014). The % of inhibition was calculated as DPPH% = {Absorbance control - [Absorbance sample/Absorbance control]}* 100

Determination of changes in chemical properties of yellowfin tuna meat

pH value

The pH was measured according to APHA (1998) using a digital pH meter (Model: Cyberscan-500, Eutech Instruments).

Total volatile basic nitrogen (TVB-N) value

TVB-N values of fish samples was determined by micro space diffusion method (Conway, 1950) based on the consumption of 0.1 M HCl and the results were expressed as mg nitrogen per 100 g (mg N 100 g^{-1}) of fish.

Tri-methyl amino nitrogen (TMA-N)

TMA-N value was determined according to the micro diffusion method (Conway, 1950). TMA-N was calculated and expressed as mg %.

Peroxide value (PV)

Fat oxidation products of fish samples were determined as per AOCS (1989) and expressed in terms of milliequivalents of peroxide per kg (meq O_2 kg^{-1}) of sample.

Thiobarbituric acid reactive substances (TBARS)

TBARS were determined according to Yerlikaya *et al*. (2015) to evaluate the oxidation stability during chilled storage and the results were expressed as TBARS value in mg of malonaldehyde per kg (mg MDA kg^{-1}) of fish sample.

Sensory evaluation of fish quality

Sensory evaluation was carried out according to the method outlined by Wu and Mao (2009). A panel of ten trained panelists assessed the sensory properties of the fish samples using a hedonic scale for the general appearance, colour, odour and overall acceptability. The different values in the scale indicated the reactions of the panelists as: 1 - extreme dislike; 2 - very much dislike; 3 - moderate dislike; 4 - slight dislike; 5 - neutral; 6 - like slightly; 7 - like moderately; 8 - like very much and 9 - like extremely. The average scores of the above four indices were used to determine the shelf life of the fish. An acceptable shelf life was indicated by a sensory score greater than 4.

Bacteriological analysis

Total aerobic plate count (TPC) was determined as per AOAC (2002). TPC of bacteria was enumerated using plate count agar, incubated at 28°C for 48 h. The results were expressed as $\log_{10}$ cfu g^{-1} of the samples.

Statistical analysis

The storage study of the experiment was conducted following a completely randomised design with five treatments and three replicates per treatment. All data were analysed by One-way ANOVA using SPSS software (ver. 18). Duncan's multiple comparison tests was used to determine the differences between the treatment means. Results were considered statistically significant at $p<0.05$.

Results and discussion

Lemon peel extract analysis

The extraction yield of antioxidants from fruit peels rely upon the solvent used for extraction. In the present study, the yield of lemon peel extract was 20.80±1.64%. Ethanol and water are the most widely utilised extraction solvents for salubrious and abundance reasons, respectively. Results of this study were more or less similar to the results reported by Singh and Immanuel (2014). Extraction with 60% ethanol yielded 16.14±1.02% of citrus extract (Viji *et al*., 2015). Ahmad *et al*. (2006) reported yield of 11.24±0.81% when citrus peel was extracted using ethanol as solvent.

Phenolics are capable of upgrading chelation of metal ions, auto-oxidation and modulation in the activity of certain enzymes (Howard *et al*., 2003). The total phenolic content of lemon peel extract in the present study was 225 mg GAE g^{-1} and this was in agreement with Ghasemi *et al*. (2009) who reported 132.2-223.2 mg GAE g^{-1}. Moure *et al.* (2001) demonstrated that both methanol and ethanol offered better results for extraction of phenolic compounds than acetone. They stated that as the polarity of the solvent increased, higher extraction yield of total soluble solids and total extractable polyphenolics was attained**.** The total phenolic content of the study was much higher than those reported by Viji *et al*. (2015) (82.8±4.3 mg GAE g^{-1}).

Flavanoids are the secondary phenolics present in plants and exhibit potential antioxidative property. The flavanoid content of the lemon peel extract in the present study was 0.9 mg QE equivalent g^{-1}. Ghafar *et al*. (2010) studied flavonoid content in different cultivars of citrus species and reported content in the range of 2.99-22.25 mg g^{-1}. Agarwal *et al.* (2012) reported that total flavonoid content in citrus peel extracts was 21.34 mg QE g^{-1}, which is much higher than the total flavanoid content in the present study. Asjad *et al.* (2013) reported a similar observation for the flavonoid content varying from 0.2-25.7 mg QE equivalent g^{-1} in six common citrus varieties of Pakistan.

During lipid oxidation, several free radicals such as OH^-, O^- and LOO of variable reactivities are formed (Jao and Ko, 2002). DPPH radical scavenging activity assay assessed the ability of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical and when it reacts with an antioxidant compound which can donate hydrogen, it is reduced to diphenyl picryl hydrazine. The scavenging activity of DPPH of lemon peel extract was 73.13%. The results were more or less similar to those of Singh and Immanuel (2014). Wanpeng *et al*. (2017) reported that DPPH values of lemon varied from 1.08 to 8.20%. There is a positive relationship between the total phenolic content and free radical scavenging activities, thus inhibiting lipid oxidation (Viji *et al.,* 2015).

Chemical analysis of yellowfin tuna meat

pH value

pH values of different experimental groups of yellowfin tuna meat samples during refrigerated storage are presented in Fig. 1. In general, pH values recorded for fresh fish is 6.7 and spoiled fish is above 7.0 (Huss, 1995). The initial pH value among different groups were 6.82±0.01, 6.60±0.10, 6.56±0.04, 6.42±0.04 and 6.34±0.05 for C, LPE1%, CH1%, LPE+CH1% and LPE+CH2% respectively. At the end of storage study, the pH of control sample was higher than that of LPE+CH1% and LPE+CH2%. The pH value gradually increased during the storage time; significant changes were found in comparison with the control after 12 days. The higher pH values in control and LPE, CH samples during storage could be attributed to the accumulation of more basic compounds (TVB-N and TMA) (Soares *et al.,* 2013). Compared to the present study, similar pH results were also reported by Liu *et al.* (2013) and Fan *et al.* (2009) for grass carp fillets and silver carp respectively during frozen storage study.

TVB-N value

Fish deterioration is a progressive proteolysis of the flesh tissue caused by the action of microorganisms and autolytic enzymes (Ocano-Higuera *et al.,* 2011). The TVB-N analysis is used as an indicator of quality in aquatic products stored at refrigerated temperatures. TVB-N includes calculation of trimethylamine, dimethylamine, ammonia and other compounds, which is chiefly from the degradation of proteins and non-protein nitrogenous compounds by activity of endogenous enzymes. TVB-N concentrations of the yellowfin tuna meat are presented in Fig. 2. In control samples, TVB-N values increased from 11.36±0.01 to 44.62±0.31 mg N 100 g^{-1} at the end of 12 days of storage ($p<0.05$). The limit of acceptability of TVB-N in fish is 35 mg N 100 g^{-1} (Huss, 1995; Jinadasa, 2014). Samples of LPE+CH1% and LPE+CH2% recorded significantly lower TVB-N value than that of the control and the samples were found acceptable till 8th (control and LPE1%), 10th (CH1%) and 12th day (LPE+CH1% and LPE+CH2%) of storage. Erkan *et al*. (2011) reported a similar observation for TVB-N value as 28.14 and 31.17 mg N 100 g^{-1} for bluefish treated with thyme and laurel essential oils at the end of storage.

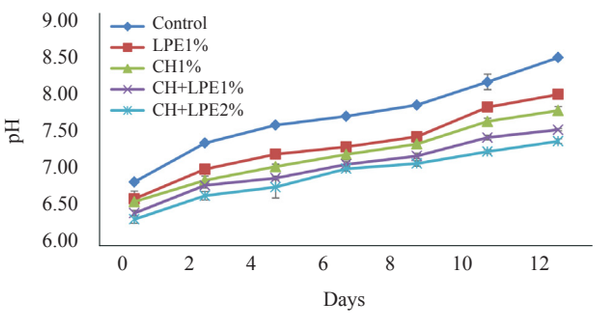

Fig. 1. pH value of yellowfin tuna meat during refrigerated storage

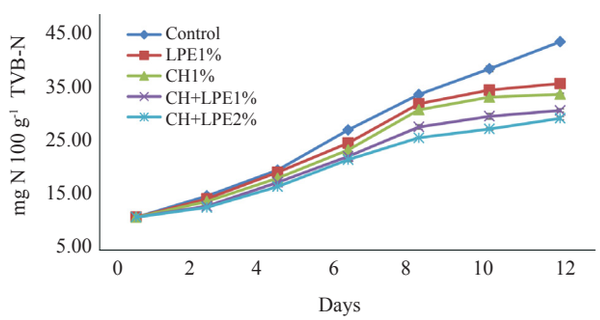

Fig. 2. TVB-N value of yellowfin tuna meat during refrigerated storage

TMA-N value

TMA-N is an important spoilage index, particularly in marine fishes. TMA-N is derived from trimethylamine oxide (TMAO) which is critical for osmoregulation in marine fish. During spoilage, TMAO is reduced to TMA by the action of bacteria. Variation in TMA-N during storage is shown in Fig. 3. TMA-N value gradually increased during the whole storage period and reached upto 7.31±0.06 mg 100 g^{-1} and 6.01±0.05 mg 100 g^{-1} in the control and LPE1% respectively after 12 days. TMA-N values for all treatments were lower than that of control samples after 10 days of storage and no significant difference was found between them. However, LPE+CH2% exhibited the lowest value of 4.51±0.11 mg 100 g^{-1}. Among the treated samples, the combined concentration of chitosan and LPE (2%) showed a very low TMA value ($p<0.05$). Sikorski *et al*. (1990) reported that the limit of TMA-N in fatty fish is 10-15 mg%. In the present study an increasing trend was observed in all

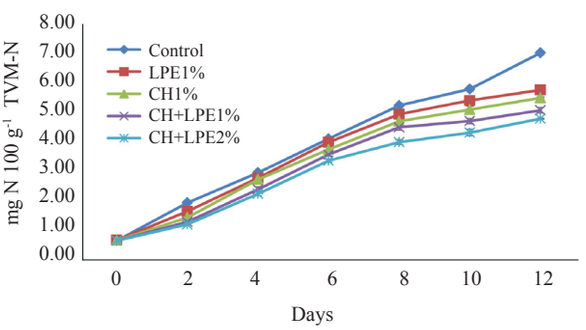

Fig. 3. TMA-N value of yellowfin tuna meat during refrigerated storage

treated samples during the refrigerated storage, but the rate of increase was significantly higher for the untreated samples than the treated samples. Jeon *et al.* (2002) and Mohan *et al*. (2012) also reported that untreated cod and sardines respectively showed significantly higher increase of TMA-N values than samples coated with chitosan. Results of the study showed that the TMA value can be lowered by using lemon peel extract in combination with chitosan.

Peroxide value (PV)

Fish contain lipid, which is susceptible to oxidation and PV measures the amount of hydroperoxides formed *i.e*., hydrocarbons, furans and other products which contribute to rancid taste in decaying fish muscle (Singleton *et al*., 1999; Tarkhasi, 2016). The variations in mean peroxide values are presented in Fig. 4. On day 1, there was no significant difference between the PV of different sample groups ($p>0.05$). As storage progressed; control, LPE+CH1% and LPE+CH2% groups showed a progressive increase in PV till 12th day of storage. The maximum PV recorded was 3.84±0.04, 3.42±0.09 and 3.29±0.03 meq O_2 kg^{-1} sample for control, LPE+CH1% and LPE+CH2% samples respectively. The values were significantly higher in control than treated samples ($p<0.05$). The ability to prevent peroxide formation was higher in LPE+CH2% due to the higher concentration of antioxidant extracts in comparison to LPE+CH1%. Similar PV results were also reported by Viji *et al.* (2015) when Indian mackerel coated with a combination of citrus peel and mint leaf extracts were stored under similar conditions. Quitral *et al*. (2009) and Bensid *et al*. (2014) also reported similar findings in Chilean jack mackerel and anchovy stored in ice with rosemary and thyme; oregano and rosemary extracts respectively. This retardation of lipid oxidation was attributed to the additional coating with chitosan, as it retarded the synthesis of oxidated primary compounds in herring, trout, cod and croaker in frozen storage as well as in ice storage (Jeon *et al.,* 2002; Ojagh *et al*., 2010a).

TBARS value

TBARS value has been universally used as an indicator for the assessment of degree of lipid oxidation. TBARS values of fish flesh are usually within the limit of 1-2 mg MDA kg^{-1} (Connel, 1995). TBARS values on day 0 was 0.30±0.01 mg MDA kg^{-1} in all the experimental groups. However, at the end of 10 days of refrigerated storage, the highest (3.03±0.02 mg MDA kg^{-1}) and lowest (2.14±0.05 mg MDA kg^{-1}) TBARS values were observed in the control and LPE+CH2% groups respectively ($p<0.05$) (Fig. 5). In the present study, lower TBARS values were observed in yellowfin tuna samples with combined coating of LPE+CH1% and LPE+CH2% than in samples coated with LPE1% and CH1% alone. This observation was similar to the results from Li *et al*. (2012; 2013) and Ojagh *et al*. (2010a). Lipid oxidation could be inhibited by coating treatments and a coating containing chitosan and tea polyphenols exhibited a slightly better effect than that of chitosan and grape seed extract. Both antioxidant and oxygen barrier properties of chitosan have been reported previously (Fan *et al.,* 2009; Ojagh *et al.,* 2010b).

Bacteriological analysis

The total aerobic plate count of yellowfin tuna meat during refrigerated storage is depicted in Fig. 6. For marine and freshwater species, the microbiological limit recommended by the ICMSF (1986) for total viable count at 30ºC is 7 log g^{-1}/log cm^{-2}. The initial TVC for all samples were approximately 5.48±0.01 log CFU g^{-1} and showed significant differences among the groups ($p<0.05$). In general, an increase in TPC was observed in all samples ($p<0.05$), however significant inhibitory effect was noticed in LPE+CH samples. Comparatively lower bacterial load was recorded in samples coated with combination of LPE and CH compared to the control samples, at the end of storage period (Fig. 6). Antibacterial effects of LPE were reported by Yamasaki *et al.*

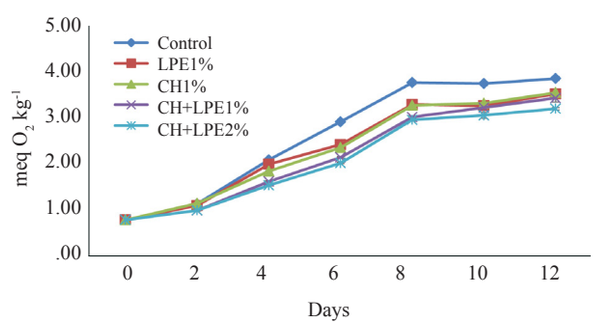

Fig. 4. PV of yellowfin tuna meat during refrigerated storage

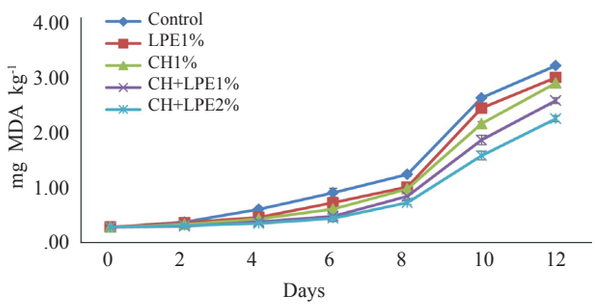

Fig. 5. TBARS value of yellowfin tuna meat during refrigerated storage

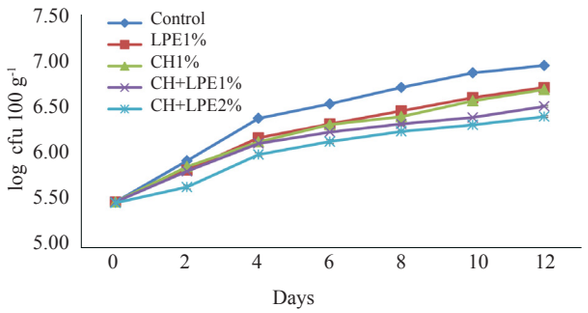

Fig. 6. Total aerobic plate count (TPC) of yellowfin tuna meat during refrigerated storage

(2007) and Dugo and and Mondello (2010). Chitosan is believed to have antimicrobial potency and has been addressed earlier (Ojagh *et al.,* 2010b; Mohan *et al.,* 2012). Growth of both Gram negative and Gram positive bacteria were suppressed by using chitosan with higher degree of deacetylation (Tsai *et al*., 2002; Qin *et al*., 2006; Huang *et al*., 2012; Mohan *et al*., 2012). Reduced bacterial growth observed in the present investigation might be due to the higher degree of deacetylation in the chitosan used. Chitosan coating acts as an oxygen barrier in products and can inhibit the growth of aerobic bacteria (Devlieghere *et al*., 2004). The present study confirms the results of Alparslan and Baygar (2017) who reported that the combined effect of chitosan and orange peel essential oil significantly reduced the bacterial load in shrimp samples under refrigerated storage.

Sensory evaluation of fish quality

The results of the sensory assessment of samples are depicted in Table 2. Samples were considered acceptable for human consumption until the sensory score reached 4 (Ojagh *et al*., 2010a). The sensory score for the control, LPE1%; CH1%; LPE+CH1% and LPE+CH2% samples declined to 4.03±0.15, 5.20±0.20, 6.17±0.29, 6.33±0.42 and 7.12±0.13 respectively after 8 days of storage. After 10 days of storage control, LPE1% and CH1% samples registered unacceptable scores. The treatments especially LPE+CH2% significantly ($p<0.05$) led to improved overall sensory score compared with control after 12 days of storage. Compared with fish samples coated with LPE1%, CH1%, LPE+CH1% and LPE+CH2%, the overall acceptability of the control samples decreased sharply from days 6 to 12 and they had significantly lower scores on days 8, 10 and 12 ($p<0.05$). The results of sensory evaluation could be correlated with high production of lipid, microbial load and products formed like ammonia, leading to off odour and off flavour which resulted in the poor score for these samples (Bazargani-Gilani *et al.,* 2015). In the present study, the fish samples treated with combination of lemon peel extract and chitosan showed higher sensory scores and showed better characteristics for odour, flavour and appearance than the control samples during the storage period. Polyphenolic compounds such as p-coumaric, ferulic and sinapic acids, narirutin as well as other constituents like hespridin and alpha-terpinene found in citrus species have been reported to impart preservative action of citrus peels (Manthey and Grohmann, 2001; Singh *et al.,* 2010). Ozyurt *et al.* (2012) reported that the addition of natural extract rosemary improved the sensory quality of *Sardinella aurita* stored under ice.

The results of the present study revealed that dipping treatment with a combination of lemon peel extract and chitosan solution significantly inhibited the occurrence of lipid oxidation, delayed biochemical quality deterioration, inhibited microbial growth and enhanced sensory qualities and shelf life of yellowfin tuna meat under refrigerated storage.

Table 1. Yield, total phenolics, flavanoids and antioxidant activity of lemon peel extract

Parameter	Value
Total yield of extract (%)	20.80±1.64
Total phenolic content (mg GAE g^{-1})	22 ±2.06
Total flavonoid content (mg QE g^{-1})	0.90±0.08
DPPH activity (%)	73.13±0.59

GAE: Gallic acid equivalent; QE: Quercetin equivalent

Table 2. Sensory evaluation results of yellowfin tuna meat during refrigerated storage

Treatment	Days of Storage						
	0	2	4	6	8	10	12
Control	8.60±0.26	7.10±0.10^{a}	6.20±0.20^{a}	5.20±0.26^{a}	4.03±0.15^{a}	3.43±0.40^{a}	2.60±0.53^{a}
LPE1%	8.63±0.15	8.07±0.12^{b}	7.33±0.31^{b}	6.17±0.29^{b}	5.20±0.20^{b}	4.20±0.26^{b}	3.43±0.40^{ab}
CH1%	8.53±0.25	8.17±0.21^{bc}	7.20±0.26^{b}	7.13±0.15^{c}	6.17±0.29^{c}	4.37±0.55^{b}	3.60±0.53^{b}
CH+LPE1%	8.50±0.10	8.47±0.15^{d}	8.13±0.12^{c}	7.20±0.26^{c}	6.33±0.42^{c}	5.60±0.53^{c}	4.50±0.50^{c}
CH+LPE2%	8.50±0.10	8.37±0.15^{cd}	8.07±0.12^{c}	7.25±0.25^{c}	7.12±0.13^{d}	6.25±0.25^{c}	5.50±0.50^{d}

Means sharing different superscripts in the same column are significantly different ($p<0.05$)

Acknowledgements

The authors wish to thank the Director, School of Industrial Fisheries, Cochin University of Science and Technology, Kerala, India for the facilities provided.

Reference

Agarwal, M., Kumar, A., Gupta, R. and Upadhyaya, S. 2012. Extraction of polyphenol, flavonoid from *Emblica officinalis*, *Citrus limon, Cucumis sativus* and evaluation of their antioxidant activity. *Orient. J. Chem*., 28: 993.

Ahmad, M. M., Salim-UR-Rehman, F. M., Iqbal-Anjum and Sultan, J. I. 2006. Genetic variability to essential oil composition in four citrus fruit species. *Pak. J. Bot.,* 38(2): 319-324.

Alparslan, Y. and Baygar, T. 2017. Effect of chitosan film coating combined with orange peel essential oil on the shelf life of deepwater pink shrimp. *Food Bioprocess Technol*., 10: 842-853. DOI10.1007/s11947-017-1862-y.

AOAC 2002. *Official methods of analyses,* 17th edn. Association of Official Analytical Chemists., Washington DC, USA, 570 pp.

AOCS 1989. *Official methods and recommended practices of the American Oil Chemists' Society*. 4th edn. Method Cd 8b-90. American Oil Chemists' Society, Champaign, Illinois, USA.

APHA 1998. *Standard methods for the examination of water and wastewater,* American Public Health Association*,* Washington DC, USA, 19 pp.

Asjad, H. M. M., Akhtar, M. S., Bashir, S., Din, B., Gulzar, F., Khalid, R. and Asad, M. 2013. Phenol, flavonoid contents and antioxidant activity of six common citrus plants. *Pak. J. Pharm. Cosmet. Sci*., 1: 1-5.

Bazargani-Gilani, B., Aliakbarlu, J. and Tajik, H. 2015. Effect of pomegranate juice dipping and chitosan coating enriched with *Zataria multiflora* Boiss essential oil on the shelf-life of chicken meat during refrigerated storage. *Innovative Food Sci. Emerg. Technol*., 29: 280-287. DOI10.1016/j.ifset.2015.04.007.

Bensid, A., Ucar, Y., Bendeddouche, B. and Ozogul, F. 2014. Effect of the icing with thyme, oregano and clove extracts on quality parameters of gutted and beheaded anchovy (*Engraulis encrasicholus*) during chilled storage. *Food Chem*., 145: 681-686. DOI: 10.1016/j.foodchem.2013.08.106.

Chaiyakosa, S., Charernjiratragul, W., Umsakul, K. and Vuddhakul, V. 2007. Comparing the efficiency of chitosan with chlorine for reducing *Vibrio parahaemolyticus* in shrimp. *Food Control*., 18: 1031-1035. DOI: 10.1016/j.foodcont.2006.06.008.

Chang, C., Yang, M., Wen, H. and Chern, J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal*., 10: 178-182.

Chaparro-Hernandez, S., Ruiz-Cruz, S., Marquez-Rios, E., Ocano-Higuera, V. M., Valenzuela-Lopez, C. C., Ornelas-Paz, J. J. and Del-Toro-Sanchez, C. L. 2015. Effect of chitosan-carvacrol edible coatings on the quality and shelf life of tilapia (*Oreochromis niloticus*) fillets stored in ice. *Food Sci. Technol. Campinas*, 35: 734-741.

Connel, J. J. 1995. *Control of fish quality*. Blackwell Science Ltd., Cambridge, UK, 241 pp.

Conway, E. 1950. *Microdiffusion analysis and volumetric error*. Crosby Lockwood and Son Ltd., London, UK.

Devlieghere, F., Vermeulen, A. and Debevere, J. 2004. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiol*., 21: 703-714. DOI: 10.1016/j.fm.2004.02.008.

Dhanavade, M. J., Jalkute, C. B., Ghosh, J. S. and Sonawane, K. D. 2011. Study antimicrobial activity of lemon (*Citrus lemon* L.) peel extract. *Br. J. Pharmacol. Toxicol*., 2: 119-122.

Diankov, S., Karsheva, M. and Hinkov, I. 2011. Extraction of natural antioxidants from lemon peels: Kinetics and antioxidant capacity. *J. Univ. Chem. Technol. Metallurgy*, 46: 315-319.

Dugo, G. and Mondello, L. 2010. *Citrus oils: composition, advanced analytical techniques, contaminants and biological activity*. CRC Press, Florida, USA.

Encarnacion, A. B., Fagutao, F., Jintasataporn, O., Worawattanamateekul, W., Hirono, I. and Ohshima, T. 2012. Application of ergothioneine-rich extract from an edible mushroom *Flammulina velutipes* for melanosis prevention in shrimp, *Penaeus monodon* and *Litopenaeus vannamei*. *Food Res. Int*., 45: 232-237. DOI10.1016/j.foodres.2011.10.030.

Erkan, N., Tosun, S. Y., Ulusoy, S. and Uretener, G. 2011. The use of thyme and laurel essential oil treatments to extend the shelf life of bluefish (*Pomatomus saltatrix*) during storage in ice. *J. Verbrauch Lebensm*., 6: 39-48. DOI10.1007/s00003-010-0587-x.

Fan, W., Sun, J., Chen, Y., Qiu, J., Zhang, Y. and Chi, Y. 2009. Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. *Food Chem*., 115: 66-70. DOI: 10.1016/j.foodchem.2008.11.060.

Ghafar, M. F., Prasad, K. N., Weng, K. K. and Ismail, A. 2010. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from *Citrus* species. *African J. Biotechnol*., 9(3): 326-330.

Ghasemi, K., Ghasemi, Y. and Ebrahimzadeh, M. A. 2009. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak. J. Pharm. Sci*., 22: 277-281.

Gomez-Estaca, J., Gimenez, B., Montero, P. and Gomez-Guillen, M. C. 2009. Incorporation of antioxidant borage extract into edible films based on sole skin gelatin or a commercial fish gelatin. *J. Food Eng*., 921: 78-85.

Howard, L. R., Clark, J. R. and Brownmiller, C. 2003. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric*., 83: 1238-1247. DOI: 10.1002/jsfa.1532.

Huang, J. Y., Chen, Q. C., Qiu, M. and Li, S. Q. 2012. Chitosan-based edible coatings for quality preservation of post-harvest whiteleg shrimp (*Litopenaeus vannamei). J. Sci. Food Agric*., 77: 491-496. DOI: 10.1111/j.1750-3841. 2012.02651.x.

Huss, H. H. 1995. Fresh fish, quality and quality changes. *FAO Fisheries series, no. 29*. Food and Agriculture Organisation, Rome, Italy, 132 pp.

ICMSF 1986. Sampling plans for fish and shellfish. In: *Sampling for microbiological analysis: Principles and scientific applications,* 2nd edn. International Commission on Microbiological Specifications for Microorganisms in Foods. University of Toronto Press, Toronto, Canada. p. 181-196.

Jao, C. L. and Ko, W. C. 2002. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. *Fish Sci*., 68: 430-435. DOI: 10.1046/j.1444-2906.2002.00442.x.

Jeon, Y. J., Kamil, J. Y. and Shahidi, F. 2002. Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. *J. Agric. Food Chem*., 50: 5167-5178. DOI: 10.1021/jf011693l.

Jinadasa, B. K. K. K. 2014. Determination of quality of marine fishes based on total volatile base nitrogen test (TVB-N). *Nat. Sci.,* 12(5): 106-111.

Jinadasa, B. K. K. K., Galhena, C. K. and Liyanage, N. P. P. 2015. Histamine formation and the freshness of yellowfin tuna (*Thunnus albacares*) stored at different temperatures. *Cogent Food Agric*., 1: 1028735. doi.org/10. 1080/23311932.2015.1028735.

Kong, M., Chen, X. G., Xing, K. and Park, H. J. 2010. Antimicrobial properties of chitosan and mode of action: a state of the art review. *Int. J. Food Microbiol*., 144: 51-63. DOI: 10.1016/j.ijfoodmicro.2010.09.012.

Kurita, K. 2006. Chitin and chitosan: functional biopolymers from marine crustaceans. *Mar. Biotechnol*., 8: 203. DOI: 10.1007/ s10126-005-0097-5.

Leclercq, C., Arcella, D. and Turrini, A. 2000. Estimates of the theoretical maximum daily intake of erythorbic acid, gallates, butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT) in Italy: a stepwise approach. *Food Chem. Toxicol.,* 38: 1075-1084. DOI: 10.1016/s0278-6915(00)00106-x.

Li, T., Hu, W., Li, J., Zhang, X., Zhu, J. and Li, X. 2012. Coating effects of tea polyphenol and rosemary extract combined with chitosan on the storage quality of large yellow croaker (*Pseudosciaena crocea*). *Food Control*., 25: 101-106. DOI: 10.1016/s0278-6915(00)00106-x.

Li, T., Li, J., Hu, W. and Li, X. 2013. Quality enhancement in refrigerated red drum (*Sciaenops ocellatus*) fillets using chitosan coatings containing natural preservatives. *Food Chem*., 138: 821-826. DOI: 10.1016/j. foodchem.2012.11.092.

Lin, D. and Zhao, Y. 2007. Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables. *Compr. Rev. Food Sci. F.,* 6(3): 60-75.

Liu, D., Liang, L., Xia, W., Regenstein, J. M. and Zhou, P. 2013. Biochemical and physical changes of grass carp (*Ctenopharyngodon idella*) fillets stored at -3 and 0°C. *Food Chem*., 140: 105-114. DOI: 10.1016/j.foodchem. 2013.02.034.

Manthey, J. A. and Grohmann, K. 2001. Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *J. Agric. Food Chem*., 49: 3262–3273. DOI: 10.1021/jf010011r>.

Maziero, G. C., Baunwart, C. and Toledo, M. C. F. 2001. Estimates of the theoretical maximum daily intake of phenolic antioxidants BHA, BHT and TBHQ in Brazil. *Food Addit. Contam.,* 18: 365-373. DOI: 10.1080/ 02652030120645.

McCune, L. M. and Johns, T. 2002. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous people of the North American boreal forest. *J. Ethnopharmacol*., 82: 197-205. DOI: 10.1016/s0378-8741(02)00180-0.

Miran, W., Nawaz, M., Jang, J. and Lee, D. S. 2016. Conversion of orange peel waste biomass to bioelectricity using a mediator-less microbial fuel cell. Sci. *Total Environ*., 547: 197-205. DOI: 10.1016/j.scitotenv.2016.01.004.

Mohan, C. O., Ravishankar, C. N., Lalitha, K. V. and Gopal, T. S. 2012. Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (*Sardinella longiceps*) during chilled storage. *Food Hydrocoll*., 26: 167-174.

Mohan, C. O., Remya, S., Murthy, L. N., Ravishankar, C. N. and Kumar, K. A. 2015. Effect of filling medium on cooking time and quality of canned yellowfin tuna (*Thunnus albacares*). *Food Control*., 50: 320-327. DOI10.1016/j. foodcont. 2014.08.030.

Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sineiro, J., Domıinguez, H. and Parajo, J. C. 2001. Natural antioxidants from residual sources. *Food Chem*., 72: 145-171. doi:10.1016/S0308-8146(00)00223-5.

Ocano-Higuera, V. M., Maeda-Martínez, A. N., Marquez-Rios, E., Canizales-Rodriguez, D. F., Castillo-Yanez, F. J., Ruiz-Bustos, E. and Plascencia-Jatomea, M. 2011. Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. *Food Chem*., 125: 49-54. doi.org/10.1016/j.foodchem.2010.08.034.

Ojagh, S. M., Rezaei, M., Razavi, S. H. and Hosseini, S. M. H. 2010a. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chem*., 120: 193-198. DOI:10.1016/j.foodchem.2009.10. 006.

Ojagh, S. M., Rezaei, M., Razavi, S. H. and Hosseini, S. M. H. 2010b. Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chem*., 122: 161-166. DOI : 10.1016/j.foodchem.2010.02.033.

Ozyurt, G., Kuley, E., Balikci, E., Kacar, Ç., Gokdogan, S. and Etyemez, M. 2012. Effect of the icing with rosemary extract on the oxidative stability and biogenic amine formation in sardine (*Sardinella aurita*) during chilled storage. *Food Bioprocess, Technol*., 5: 2777-2786. DOI: 10.1007/s11947-011-0586-7.

Pathak, P. D., Mandavgane, S. A. and Kulkarni, B. D. 2017. Fruit peel waste: Characterisation and its potential uses. *Curr. Sci*., 113: 1-11.

Qin, C., Li, H., Xiao, Q., Liu, Y., Zhu, J. and Du, Y. 2006. Water-solubility of chitosan and its antimicrobial activity. *Carbohydr. Polym*., 63: 367-374.

Qiu, X., Chen, S., Liu, G. and Yang, Q. 2014. Quality enhancement in the Japanese seabass (*Lateolabrax japonicas*) fillets stored at 4°C by chitosan coating incorporated with citric acid or licorice extract. *Food Chem*., 162: 156-160.

Quitral, V., Donoso, M. L., Ortiz, J., Herrera, M. V., Araya, H. and Aubourg, S. 2009. Chemical changes during the chilled storage of Chilean jack mackerel (*Trachurus murphyi):* effect of a plant extract-icing system. *LWT-Food Sci, Technol*., 42: 1450-1454.

Rao, B. M., Jesmi, D. and Viji, P. 2017. Chilled storage of *Pangasianodon hypophthalmus* fillets coated with plant oil-incorporated alginate gels: Effect of clove leaf, clove bud, rosemary and thyme oils. *J. Aquat. Food Prod. Technol.,* 26: 744-755.

Renur, N. M., Haryadi, Y., Darmawati, E. and Kapelle, I. B. D. 2016. Application of chitosan and Chinese lemon extract (*Citrus mitis*) based edible coating on tuna fillet. *J. Food Nutr. Sci.,* 4(2): 29-33.

Sagoo, S., Board, R. and Roller, S. 2002. Chitosan inhibits growth of spoilage microorganisms in chilled pork products. *Food Microbiol*., 19: 175-182. doi.org/10.1006/fmic.2001.0474.

Shahidi, F. 2004. Functional foods: their role in health promotion and disease prevention. *J Food Sci*., 69: 146-149. doi. org/10.1111/j.1365-2621.2004.tb10727.x.

Sikorski, Z. E., Kolakowska, K., Burt, J. R. 1990. Post-harvest, biochemical and microbial changes. In: Sikorski, Z. E. (Eds.), *Seafood: Resources, nutritional composition and preservation*. CRC Press Inc., Boca Raton, Florida, USA, p. 55-75. doi.org/10.1111/j.1365-2621.2004.tb10727.x.

Singh, A., Sharma, P. K. and Garg, G. 2010. Natural products as preservatives. *Int. J. Pharm. Biol. Sci*., 1: 601-612.

Singh, S. and Immanuel, G. 2014. Extraction of antioxidants from fruit peels and its utilisation in paneer. *J. Food Process Technol*., 5: 2-5 doi:10.4172/2157-7110.1000349.

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.,* 299: 152-178.

Soares, N. M., Mendes, T. S. and Vicente, A. A. 2013. Effect of chitosan-based solutions applied as edible coatings and water glazing on frozen salmon preservation – A pilot scale study. *J. Food Eng*., 119: 316-323. doi.org/10.1016/j. jfoodeng. 2013.05.018.

Tarkhasi, A. 2016. Effect of edible coating containing pomegranate peel extract on quality and shelf life of silver carp (*Hypophthalmichthys molitrix*) fillet during refrigerated storage. *J. Food Ind. Microbiol*., 2: 112. doi: 10.4172/2572-4134.1000112.

Tsai, G., Su, W., Chen, H. and Pan, C. 2002. Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. *Fish Sci*., 68: 170-177. doi.org/10.1046/j.1444-2906.2002.00404.x.

Viji, P., Binsi, P. K., Visnuvinayagam, S., Bindu, J., Ravishankar, C. N. and Gopal, T. K. S. 2015. Efficacy of mint (*Mentha arvensis*) leaf and citrus (*Citrus aurantium*) peel extracts as natural preservatives for shelf life extension of chill stored Indian mackerel. *J. Food Sci. Technol*., 52: 6278-6289. DOI: 10.1007/s13197-015-1788-1.

Wanpeng, X. I., Zheng, Q., Juanfang, L. U. and Junping, Q. U. A. N. 2017. Comparative analysis of three types of peaches: Identification of the key individual characteristic flavor compounds by integrating consumers' acceptability with flavor quality. *Hortic. Plant J*., 3: 1-12.

Wu, T. and Mao, L. 2009. Application of chitosan to maintain the quality of kamaboko gels made from grass carp (*Ctenopharyngodon idella*) during storage. *J. Food Process Preserv*., 33: 218-230. doi.org/10.1111/j.1745-4549. 2008. 00264.x.

Yamasaki, Y., Kunoh, H., Yamamoto, H. and Akimitsu, K. 2007. Biological roles of monoterpene volatiles derived from rough lemon (*Citrus jambhiri* Lush) in citrus defense. *J. Gen. Plant Pathol*., 73: 168-179. DOI:10.1007/s10327-007-0013-0.

Yerlikaya, P., Ucak, I., Gumus, B. and Gokoglu, N. 2015. Citrus peel extract incorporated ice cubes to protect the quality of common pandora. *J, Food Sci, Technol*., 52: 8350-8356. . doi: 10.1007/s13197-015-1942-9.

Yuan, G., Lv, H., Tang, W., Zhang, X. and Sun, H. 2016. Effect of chitosan coating combined with pomegranate peel extract on the quality of Pacific white shrimp during iced storage. *Food Control*, 59: 818-823. DOI: 10.1016/j. foodcont.2015.07.011.

Date of receipt : 05.07.2019

Date of acceptance : 24.01.2020