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Effect of Chitosan on Biochemical, Microbiological and Sensory Characteristics of Restructured Products from Pangasius (*Pangasianodon hypophthalmus*)

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Abstract

In India, pangasius (*Pangasianodon hypophthalmus*) farming is gaining importance among the farmers due to its fast growth and better survival rate. In the present study, restructured products were prepared from pangasius mince in four different formulations by using pangasius fish mince, corn starch (4%) and chitosan (0.75%). Formulation containing only corn starch (4%) served as control. Shelf life of the products was evaluated under chilled (2ºC) condition up to 17 days. Biochemical quality parameters viz., total volatile base nitrogen (TVB-N), free fatty acid (FFA) and peroxide value (PV), thiobarbituric acid (TBA) value were within acceptable limits in chitosan treated samples. Control sample was acceptable only upto 6 days compare to 12 days in chitosan treated samples.

Keywords: Chilled storage, chitosan, pangasius, fish mince, restructured products.

Introduction

There is an increasing demand for fish and fishery products throughout the world due to its health benefits as fish contain all essential amino acids, fatty acids, vitamins and minerals (Elizabeth, 2013). The current trend in fish processing industry is to introduce novel fish products based on fish mince that are stable, acceptable and nutritious to meet international standards. Surimi and restructuring technology offers scope for novel products by incorporating an array of additives to improve the mechanical and functional properties. Low-value species and filleting remains could be transformed into high-value products by restructuring technology (Borderias et al., 2005). The restructuring process allows the acquisition of products with high commercial value. In India, unlike marine fish, freshwater fish is usually marketed for consumption in fresh condition.

Pangasius (*Pangasianodon hypophthalmus)* or basa catfish is one of the fast growing aquaculture species. Due to high demand in the international market, large numbers of farmers in India are engaged in culturing pangasius fish. It is mostly sold as fresh fish or frozen fillets. Until now little or few efforts have been made to produce value-added products from pangasius. Processing of pangasius into value-added battered and breaded products enhance their acceptability and market value. Food hydrocolloids have been proposed to improve the mechanical and functional properties of surimi and restructured fish gel. The importance of chitosan as a food additive is increasing due to its antioxidant and antimicrobial activities (Kamil et al., 2002; Mohan et al., 2012). Chitosan (â-(1,4)-2- amino-2-deoxy-D-glucopyranose) is the deacetylated form of chitin, which has been reported to have a number of functional properties that make it technically and physiologically useful as a kind of dietary fibre (Borderias et al., 2005). Chitosan may retard the lipid oxidation and inhibit the growth of spoilage bacteria in meat during storage. There is a considerable interest in using additives along with minced fish to extend its storage life. To the best of our knowledge, no literature is so far available on the effect of chitosan incorporation on the quality of restructured products from pangasius fish mince. The present study was undertaken to prepare restructured products from pangasius mince with

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addition of chitosan and to evaluate the effectiveness of chitosan in extending shelf life of products under chilled storage.

Materials and Methods

Pangasius with an average weight of 1.3±0.25 kg and length 48±1.5 cm were procured from Aquafarm at Kodungalloor near Cochin, India and brought to the laboratory, at CIFT, Cochin in iced condition (1:1 fish to ice ratio) in insulated boxes. All the chemicals and glassware used in the study were of analytical grade. Chitosan was prepared from the shells of *Metapenaeus dobsoni* by the method of Nair and Madhavan (1974). The degree of deacetylation of the chitosan was determined as described by Shigemassa et al. (1996).

Fish were dressed and washed with potable water. Filleting and de skinning were done manually. Mincer (SIMARN, India) was used for production of fish mince. Four different formulations (C1, C2, T1 & T2) made for product preparation are given in Table 1. Ingredients for the preparation of restructured products were optimized and set on the basis of preliminary sensory analysis (data not shown). Before preparation of products, chitosan was dissolved in known volume of 1% acetic acid solution and was added to surimi. Ingredients were mixed using a silent cutter (SCHARFFEN, Germany). The mixture was kept under refrigeration (2-3ºC) for 20 min for setting. Then it was spread (1.5 cm thickness) on an aluminium tray (30 x 30 cm) smeared with little oil to avoid sticking and steam cooked for 30 min. The cooked mincewas cooled and cut into square shapes (size; 3 x 3 cm;). After battering and breading, five to six pieces weighing 100 ± 5 g were packed in high impact polypropylene (HIPP) trays (size: 12.5 cm x 8.5 cm x 2.5 cm) of 0.88 mm thickness (KL Thermoformers, New Delhi, India). The trays were sealed using tray sealer (Vac-Star: Model no. S220 MP, Switzerland) and stored in chilled condition (2°C). Formulations C1 & C2 served as control. Samples were drawn at regular intervals and were evaluated for their biochemical, microbiological and sensory characteristics.

Proximate composition was analyzed by the method of AOAC (2005). pH of homogenized samples was measured using a calibrated glass electrode pH meter (Cyberscan 510; Eutech Instruments, Singapore). Total volatile base nitrogen (TVB-N) was determined by Conway micro-diffusion method (Conway, 1950). Free fatty acid (FFA) and peroxide value (PV) was evaluated according to AOAC (2005) method. Thiobarbituric acid (TBA) value was determined as described by Tarladgis et al. (1960). Fatty acid composition was determined by the method of AOAC (2005).

Total plate count and *Staphylococcus aureus* count were determined by the method of FAO (1992). Enterobacteriaceae and *Escherichia coli* were evaluated according to BAM (2002). Enterococci were estimated by the method of Koutsoumanis & Nychas (1999).

Sensory analysis of restructured products was done by six trained panelists of Fish Processing Division of ICAR-CIFT, Cochin. The restructured products were fried for 3 min and cooled for 2 min before serving to the panellists.. The panellists were asked to assign a score of 1-9 as prescribed by Meilgaard et al. (1999). The sensory attributes evaluated were appearance, colour, flavour, taste and overall acceptability. A high score (9–7) was given to the product with no off-odours, a score 5 to fish with a flat and neutral odor and scores below 5 to fish with off-odors. An overall acceptability score of below 5 corresponded to unacceptable quality.

The data obtained were analyzed by running one way analysis of variance (ANOVA) using statistical

Table 1. Ingredient compositions used for the preparation of restructured products

Treatment	Mince (g)	Salt (g)	Sodium Tri Polyphosphate (g)	Corn starch (g)	Chitosan (g)
C1	100	1	0.25	4.0	-
C2	100	1	0.25	-	-
T1	100	1	0.25	-	0.75
T2	100	1	0.25	4.0	0.75

Where, C1 & C2- Control; T1 & T2 - Chitosan incorporated

package for social science (SPSS) software version 16.0. (SPSS Inc, Chicago, IIIinois, USA). All mean separations were carried out by Duncan multiple range test using the significance level of 95% ($p<0.05$).

Results and Discussion

In the present study, pangasius fish mince had 77.96±0.12% moisture, 16.58±0.35% protein, 2.59±0.1% lipid and 1.5±1.2% ash on a wet weight basis. The results from the study are in agreement with similar results observed for fresh pangasius fish muscle (Orban et al., 2008; Viji, et al., 2014). Fatty acid profile revealed that, higher percentage of fatty acids present in the pangasius fish mince were saturated fatty acids (42.57%) followed by monounsaturated fatty acids (40.11%) and polyunsaturated fatty acids (17.45%) (Table 2) which was similar to that observed by Men et al. (2005) for pangasius fish meat.

Chitosan prepared in this study had 8.45±32% moisture, 0.82±0.05% protein and 0.86±0.06% ash. Colour of chitosan showed *L**, *a** and *b** values of 77.43±0.30, 1.96 ±0.04, 18.81±0.15 respectively. The degree of deacetylation, which is one of the important properties of chitosan, deciding its application (Baxter et al. 1992), was found to be 90%. These results indicate that the chitosan prepared from the shell of *Metapenaeus dobsoni* confirms to the standards of food grade chitosan (Dayong, 2012).

In general, the moisture content of all the samples showed an increasing trend during chilled storage. Rathod & Pagarkar (2013) also observed slight variation in moisture content of fish cutlet stored at refrigerated condition. In the present study, pH of restructured products showed an increasing trend during storage (Table 3). Binsi et al. (2007) reported that increase in pH during storage may be due to the production of amines and other volatile bases by the autolytic and microbial action on protein and other compounds. It was observed that pH was less in T1 (6.20-6.59) and T2 (6.11-6.52) samples than C1 (6.30- 6.68) and C2 (6.32-6.62) samples whichmay be due to the incorporation of chitosan (which is pre dissolved in 1% acetic acid) in T1 and T2 sample.

Total volatile base nitrogen content has been traditionally used as a quality indicator of fish and fishery products stored in ice. In the present study, total volatile base nitrogen (TVB-N) showed increasing trend and was found to be higher in C2 (1.12 - 12.5 mg 100 g^{-1}) and C1 (1.0 – 11.9 mg 100 g^{-1}) samples than the chitosan incorporated (T1 and T2) samples (6.5- 7.5 mg 100 g^{-1}) during storage (Fig 1.). The increase in TVB-N during storage is a consequence of liberation of basic compounds by microbial activity on protein and non-protein nitrogenous compounds (Ninan et al. 2008; Viji et al., 2014). Fan et al. (2009) observed reduction of the TVB-N values in chitosan coated silver carp. They also reported

Table 2. Fatty acid compositions of pangasius (*Pangasianodon hypophthalmus*) fish mince

Fatty acid	Peak Area (%)
C 6:0	0.22
C 8:0	0.13
C 10:0	0.21
C 11:0	0.30
C 12:0	2.12
C 14:0	2.46
C 16:0	26.5
C 17:0	0.80
C 18:0	4.50
C 20:0	0.12
C 21:0	2.15
C 23:0	1.64
C 24:0	1.42
Σ SAFA	42.57
C 16:1	2.25
C 17:1	0.14
C 18:1	29.68
C 20:1	4.33
C 22:1	1.12
C 24:1	2.59
Σ MUFA	40.11
C 18:2	8.08
C 18:3	0.75
C 20:2	0.36
C 20:3	4.88
C 20:4	0.38
C 20:5	0.74
C 22:4	1.50
C 22:6	0.76
Σ PUFA	17.45

Table 3. Changes in moisture and pH of restructured products during chilled storage

Parameter/ days	Treatment	0	3	6	9	12	17
Moisture(%)	C1	59.20±0.2^{b}	59.94±0.2^{c}	60.74±0.2^{e}	60.67±0.30^{e}	60.20±0.20^{d}	58.49±0.55^{a}
	C2	61.80±0.15^{e}	60.37±0.3^{b}	60.62±0.20^{c}	60.26±0.25^{a}	60.66±0.10^{d}	63.88±0.45^{f}
	T1	61.69±0.20^{c}	60.11±0.1^{a}	63.17±0.15^{e}	61.19±0.20^{b}	62.26±0.15^{d}	64.55±0.65^{f}
	T2	61.99±0.10^{b}	60.97±0.32^{a}	63.78±0.10^{c}	61.68±0.15^{b}	62.10±0.12^{b}	60.67±0.42^{a}
pH	C1	6.30±0.05^{a}	6.33±0.04^{a}	6.42±0.01^{b}	6.48±0.05^{c}	6.59±0.02^{d}	6.62±0.02^{e}
	C2	6.32±0.07^{a}	6.41±0.02^{b}	6.44±0.05^{c}	6.57±0.02^{d}	6.64±0.01^{d}	6.68±0.03^{e}
	T1	6.20±0.02^{a}	6.37±0.01^{b}	6.44±0.04^{c}	6.48±0.03^{d}	6.51±0.02^{e}	6.59±0.05^{f}
	T2	6.11 ±0.01^{a}	6.16±0.02^{b}	6.22±0.02^{c}	6.39±0.02^{d}	6.44±0.05^{e}	6.52±0.01^{f}

Results are mean ± SD; (n = 3); Values within a row with different superscript letters are significantly ($p<0.05$) different. C1 & C2- Control; T1 & T2- Chitosan incorporated

that reduction in TVB-N values of samples might be on account of chitosan incorporation which also resulted in a reduction of the bacterial population and a decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds. The TVB-N value of < 20 mg N 100 g^{-1} sample was considered fresh, < 30 mg N 100 g^{-1} sample was acceptable and >40 mg N 100 g^{-1} sample was not suitable for consumption (Mendes et al., 2005). A rejection limit of 25 mg N100 g^{-1} has been proposed for fresh water fish (Gimenez et al., 2002). In the present study, none of the samples crossed the rejection limit during chilled storage.

Free fatty acid (FFA) value of restructured fish products showed an increasing trend from 0.02 to 4.5% of oleic acid (Fig 2.) which indicated that lipid hydrolysis continued even at low temperatures. The formation of FFA proceeds during storage is probably due to the action of lipases and phospholipases (Aubourg et al., 2010). Peroxide value showed an increasing trend during storage (Fig. 3) which was similar to results obtained by Ninan et al. (2008). However, C2 samples exhibited higher peroxide value. It may be due to ingredient composition i.e C2 sample had only salt and STPP along with fish mince. It was observed that restructured products prepared with chitosan had less peroxide value (T1 - 2.58 milliequivalent O_2 Kg^{-1}; T2 - 2.45 milliequivalent O_2 Kg^{-1}) than control (C1 - 3.25 milliequivalent O_2 Kg^{-1}; C2 - 5.1 milliequivalent O_2 Kg^{-1}) samples on 17th day. Kamil et al. (2002) reported that chitosan possess antioxidant and antibacterial capacity and it may retard lipid oxidation and inhibit the growth of spoilage bacteria in meat during storage. A steady increase in the TBA value (0.82 to 3.74 mg malonaldehyde kg^{-1}) was observed during chilled storage (Fig. 4). The maximum TBA value that indicates the good quality of the fish frozen, chilled or stored with ice is 5 mg malonaldehyde kg^{-1}.

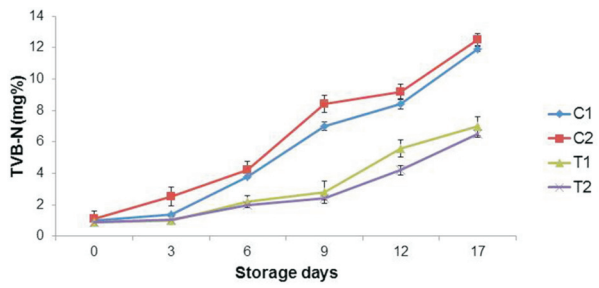

Fig. 1. Changes in TVB-N values of restructured products during chilled storange

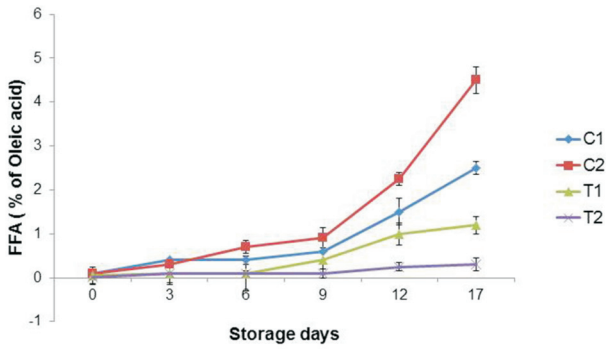

Fig. 2. Changes in FFA values of restructured products during chilled storange

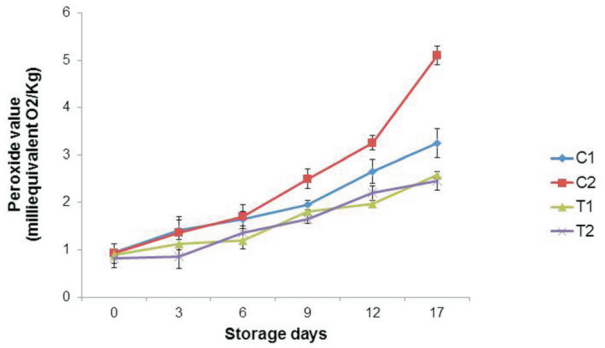

Fig. 3. Changes in peroxide values (PV) of restructured products during chilled storange

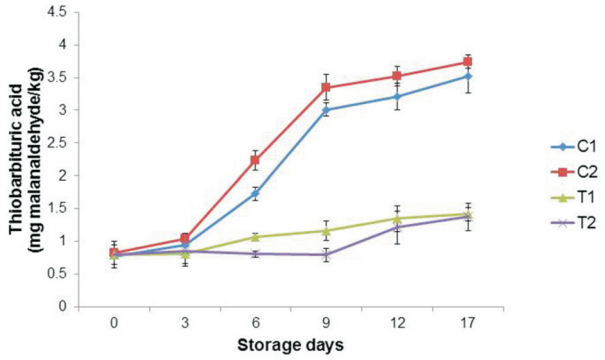

Fig. 4. Changes in thiobarbituric acid (TBA) values of restructured products during chilled storange

Further, the fish can be consumed if the TBA value is up to a level of 8 mg malonaldehyde kg^{-1} (Adenike, 2014). In the present study, chitosan treated sample (T1 & T2) showed less TBA value (1.38-1.42 mg malonaldehyde kg^{-1}) than C1 (3.52 mg malonaldehyde kg^{-1}) and C2 (3.74 mg malonaldehyde kg^{-1}) samples. Chitosan may retard lipid oxidation by chelating ferrous ions present in the system, thus eliminating their per oxidant activity or their conversion to ferric ion (Xue et al., 1998). Park et al. (2004) reported that chitosan with higher degree of deacetylation have the highest antioxidant activity. The chitosan used in the present study had a high degree of deacetylation (90%) and effectively retarded the lipid oxidation.

The initial total bacterial counts in C1 and C2 samples were 5.38 log cfu $g^{-1}$and 5.35 log cfu g^{-1} respectively. On further storage, the count continued to increase gradually and reached a level of 7.32 and 7.65 log cfu g^{-1} respectively on 9th day (Fig. 5).

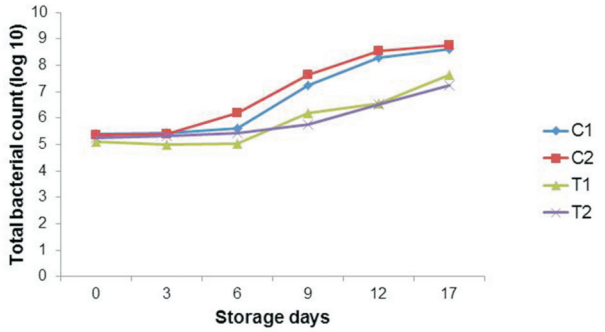

Fig. 5. Changes in total bacterial count of restructured products during chilled storange

A 7 log cfu g^{-1} is considered as acceptable limit for restructured fish product (Jay, 1996). It was observed that C1 and C2 samples crossed the acceptable limit on 9th day, while, T1 (7.65 log cfu g^{-1}) and T2 (7.25 log cfu g^{-1}) samples crossed the limit on 17th day. Results indicated that the changes in total bacterial counts were significantly ($p<0.05$) higher for control (C1 & C2) compared to chitosan-incorporated samples. Several authors reported that antimicrobial activity of chitosan is due to disruption of the lipopolysaccharide layer of the outer membrane of gram-negative bacteria and to its function as a barrier against oxygen transfer (Kanatt et al., 2013; Jeon et al., 2002). Qin et al. (2006) reported that chitosan with low and medium molecular weight and high degree of deacetylation of over 80% is able to suppress the growth of both gram-positive and gram-negative bacteria. The reduced microbial growth in chitosan-incorporated product in the present study could be attributed to the high degree of deacetylation (90%). In the present study, Enterobacteriaceae, *E. coli*, *S. aureus* and enterococci were not detected in any ofthe products (C1, C2, T1 & T2).

Sensory evaluation results showed good appearance of the products was good throughout storage. All the products had an overall acceptablity score of 6.5 to 8.0 up to 6th day. There was a decreasing trend in overall acceptability after 6th day (Fig. 6). Further, products prepared without chitosan (C1 & C2) had an overall acceptability score of 3.8 - 4.1 due to off- odour on 9th day and were rejected. This may be due to formation of some volatile low molecular weight compounds, lipid oxidation and protein degradation during storage (Pawar, 2011). However, the deterioration was slow for chitosan-incorpo-

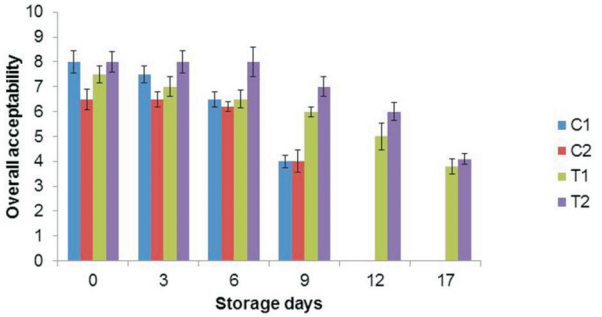

Fig. 6. Changes in overall acceptability of restructured products during chilled storange

rated samples compared to control. Chitosan incorporation showed a significant effect on all the attributes ($p < 0.05$) and the products were on 17th day with the sensory score of 3.8 and 4.1. Mohan et al. (2012) observed similar results for chitosan coated sardine fillets. Results indicated that there was significant difference ($p < 0.05$) in overall acceptability of T2 and T3 samples. In T2 sample corn flour was not added which resulted in product juiciness and reduced the score of over all acceptability of the product.

It can be concluded that products coated with chitosan showed reduced lipid oxidation and extended shelf life of products chilled storage. Sensory and microbiological analysis revealed that products prepared without chitosan was acceptable up to 6 days and was rejected on 9th day, whereas chitosan incorporated products were acceptable up to 12th day and was rejected on 17th day. However, products prepared with chitosan and corn starch showed higher overall acceptability (T2) than products with chitosan only (T1). Further investigations are required to determine the changes in textural properties of the products during chilled storage. Results from the study, indicate the possible application of chitosan as an additive to fish mince based food systems as a natural ingredient to prevent lipid oxidation and extend shelf life of the products.

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References

Adenike, O.M. (2014) The Effect of Different Processing Methods on the Nutritional Quality and Microbiological Status of Cat Fish (*Clarias lezera*). J. Food Process. Technol. doi.org/10.4172/2157-7110.1000333

AOAC (2005) Official Methods of Analysis, 15th edn., Association of Official Analytical Chemists, AOAC International, Washington DC.

Aubourg, S., Tabilo-Munizaga, G., Reyes, J., Rodriguez, A. and Perez-Won, M. (2010) Effect of high pressure treatment on microbial activity and lipid oxidation in chilled coho salmon. Eur. J. Lipid Sci Tech. 112: 362-372.

BAM (2002) Enumeration of *Escherichia coli* and the Coliform Bacteria. In: Bacteriological Analytical Manual, Chapter 4; United States Food and Drug Administration.

Baxter, A., Dillon, M., Taylor, K. D. A. and Roberts, G. A. F. (1992) Improved method for I.R. determination of the degree of N-acetylation of chitosan. Int. J. Biol. Macromol. 14, (3): 166-169.

Binsi, P.K., Shamasundar, B.A. and Dileep, A.O. (2007) Physico-chemical and functional properties of proteins from green mussel (*Perna viridis*) during ice storage. J. Sci. Food Agric. 87: 245-254.

Borderias, A.J., Sanchez I.A. and Perez-Mateos, M. (2005) New applications of fibres in foods: Addition to fishery products. Trends Food Sci. Tech. 16: 458-465.

Conway, E.J. (1950) Micro-diffusion analysis and volumetric error. 5th edn., Lockwood and Son Ltd, London, pp. 467-472.

Dayon, T. (2012) From chitin to chitosan. In: Chitosan based hydrogels: Functions and Applications. Kangde Y, Junjie L, Fanglian Y, Yuji Y (Eds), Taylor and Francis Group, Boca Raton Florida, pp. 1-39.

Elizabeth, K. L. (2013) Health benefits of seafood; Is it just the fatty acids? Food Chem. 140: 413-420.

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.

FAO (1992) Manual of food quality control, 4. Rev.1. Microbial analysis. FAO Food and Nutrition paper. Food and Agriculture Organization of the United Nations, Rome.

Folch, J., Lees, M. and Sloane-stanley, G.H. (1957) Simple method for purification and isolation of lipid (total) from animal tissue. J. Biol Chem. 225: 492-495.

Gimenez, B., Roncales P. and Beltran, J.A. (2002) Modified atmosphere packaging of filleted rain bow trout. J. Sci. Food Agri. 82: 1154-1159.

Jay, J.M. (1996) Modern food microbiology. 4th edNew Delhi: CBS Publishers and Distributors.

Kamil, J.Y.V.A., Jeon, Y.J. and Shahidi, F. (2002) Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (*Clupea harengus*). Food Chem. 79: 69–77.

Kanatt, R.S., Rao, M.S., Chawla, S.P. and Arun Sharma. (2013) Effects of chitosan coating on shelf-life of ready-to-cook meat products during chilled storage. LWT - Food Sci. Technol. 53: 321-326.

Koutsoumanis, G.K. and Nychas, J.E. (1999) Chemical and sensory changes associated with microbial flora of Mediterranean bogue (*Boops boops*) stored aerobically at 0, 3, 7, and 10°C. Appl. Environ. Microbiol. 65: 698-706.

Meilgaard, M., Civille, G. V. and Carr, B. T. (1999). Sensory evaluation techniques (3rd ed.). Boca Raton, Fla: CRC Press, pp. 387.

Mendes, R., Gonçalves, A., Pestana, J. and Pestana, C. (2005) Indole production and deepwater pink shrimp (*Parapenaeus longirostris*) decomposition. J. Eur. Food Res. Technol. 214: 125-130.

Men, L. T., Thanh, V. C., Hirata, Y. and Yamasaki, S. (2005) Evaluation of the genetic diversities and the nutritional values of the Tra (*Pangasius hypophthalmus*) and the Basa (*Pangasius bocourti*) catfish cultivated in the Mekong river delta of Vietnam. Asian–Australasian J. Animal Sci. 18: 671-676.

Mohan, C.O., Ravishankar, C.N., Lalitha, K.V. and Gopal, S.T.K. (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.

Nair, K.G.R. and Madhavan, P. (1974) Utilization of prawn waste-Isolation of chitin and its conversion to chitosan. Fish. Technol. 11: 60.

Ninan G., Bindu, J. and Joseph, J. (2008) Frozen storage studies of minced based products developed from tilapia (*Oreochromis mossambicus*, Peter 1852). Fish. Technol. 45: 35-42.

Park, P. J., Je, J. Y. and Kim, S. K. (2004) Free radical scavenging activities of differently deacetylated chitosans using an ESR spectrometer. Carbohyd. Polymer. 55: 17-22.

Pawar, P.P. (2011) Preparation of battered and breaded product from freshwater fish *(Catla catla)*. M.F.Sc thesis., Konkan Krishi Vidyapeeth, Dapoli, Maharashtra state, India

Qin, C., Li, H., Xiao, Q., Liu, Y., Zhu, J. and Du, Y. (2006) Water-solubility of chitosan and its antimicrobial activity. Carbohyd. Polymer. 63: 367-374.

Rathod, N. and Pagarkar, A. (2013) Biochemical and sensory quality changes of fish cutlets, made from pangasius fish (*pangasianodon hypophthalmus*), during storage in refrigerated display unit at -15 to -18°C. Int. J. of Food, Agri. Vet. Sci. 3: 1-8.

Shigemassa, Y., Matsuura, H. and Sashiwa, H. (1996) Evaluation of different ratios from infrared spectroscopy for analysing the degree of acetylation in chitin. Int. J. Biol. Macromol. 18: 237-242.

Tarladgis, G. B., Watts, M. B. and Younathan, T. M. A. (1960). Distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soci. 37: 44-50.

Viji, P., Tanuja, S., George Ninan, Zynudheen,.A.A. and Lalitha, K.V. (2014) Quality characteristic and shelf life of sutchi cat fish (*Pangasianodon hypophthalmus*) steaks during refrigerated storage. Int. J. Agric. Food sci. Tech. 5: 105-116.

Xue, C., Yu, G., Hirat, T., Terao, J. and Lin, H. (1998) Antioxidative activities of several marine polysaccharides evaluated in a phosphatidylcholine– liposomal suspension and organic solvents. Biosci. Biotech. Biochem. 62: 206-209.