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Effect of Green tea Extract on the Quality of Fish Nuggets during Chilled Storage

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Abstract

In the present study, fish nuggets were prepared from pink perch (*Nemipterus japonicus*) mince and their qualities were evaluated under chilled (2°C) storage. Pink perch mince had 81.46±0.25% moisture, 17.44± 0.20% protein, 0.85±0.05% fat and 1.02±0.02% ash. Fish nuggets were prepared in three different formulations by incorporating corn starch (4%), green tea extract (0.1%) and BHT (0.01%) in to fish mince referred to as: Control, GTE and BHT respectively. Biochemical and microbiological qualities of fish nuggets were evaluated up to 17th day. Results showed an increasing trend in thiobarbituric acid (TBA) values during storage. However, sample containing green tea extract had a lower TBA value (0.34±0.01 to 0.44±0.03 mg MDA kg^{-1}) than BHT incorporated sample (0.42±0.01 to 0.80±0.02 mg MDA/kg) and control (0.82± 0.02 to 2.14±0.01 mg MDA kg^{-1}). Similar trend was also observed for free fatty acids, peroxide value (PV), total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) content. Texture analysis showed an increasing trend in hardness of all samples during storage. *L** value showed a decreasing trend in (59.59±1.2 to 56.95±0.95) green tea extract incorporated sample. Based on the sensory and microbial analysis, control had a shelf life of 12 days whereas, products incorporated with green tea extract and BHT had an extended shelf life up to 15 days.

Keywords: Green tea extract, fish nugget, quality, chilled storage

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Introduction

Recently, food sector has shown an increasing demand for restructured meat products, such as fish finger, nuggets, etc. (Resurreccion, 2003). Restructure process offers many opportunities to the food industry for developing novel food products. Fish mince is one of the major raw material for developing value added seafood products and it offers flexibility in product formulation and texture modification. Fish mince gets spoiled faster than whole fish or fillet, so it has to be preserved properly to maintain good quality (Joaquin et al., 2008) for developing fish products. Fish products prepared from fish mince includes fish balls, fish fingers, cutlets etc. There is an increased demand for restructured fish products such as nuggets. Restructure process are used to obtain novel products using an array of additives to improve the mechanical and functional properties. The process of grinding and mixing of fish meat and ingredients lead to more susceptibility towards lipid oxidation which can be reduced by adding antioxidants. Although synthetic additives have been widely used in the food industry to reduce lipid oxidation and microbial activity, there is increased customer demand for antioxidants and antimicrobial agents from natural sources due to the harmful effect of synthetic antioxidants. The application of natural extract to reduce lipid oxidation has been studied in fish fillet and fish mince (Aubourg et al., 2004; Serdaroglu & Felekoglu, 2005). Green tea extract has been reported to have antioxidant and antimicrobial activities. It has been reported that green tea mainly consists of polyphenols (~90%), amino acids (~7%), theanine, proanthocyanidins, and caffeine (~3%) (Li et al., 2018) Green tea extract consists of excellent source of polyphenols, which are powerful alternative to synthetic antioxidants. Being natural, they are typically less harmful and appear to have an equivalent effect upon the inhibition of oxidation

(Manea et al., 2014). Currently, there is increasing demand for green tea extract incorporated food products by the consumer due to its beneficial health effects. Several researchers have studied the effect of green tea extract in different food products like bread, biscuit and some meat products (Wang & Zhou, 2004; Lavelli et al., 2010; Mitsumoto, et al., 2005). The present study was aimed to analyse the effect of green tea extract on the biochemical, microbial and sensory quality of fish nuggets and to determine the shelf life of fish nuggets under chilled storage (2°C).

Materials and Methods

Fresh pink perch (*Nemipterus japonicus*) were procured from fish market and it was deiced and cleaned with potable water. Fishes were dressed, filleted and mince were prepared by using mincer (SIRMAN, India) and it was used for fish nuggets preparation. All chemicals and glasswares used for the study were analytical grade. Green tea (*Camellia sinensis*) extract was procured from Perennial Lifesciences Pvt. Ltd., Delhi, India. The green tea extract contained the following components: Polyphenols-95%; Catechin- 75% ; Epigallocatechin gallate (Egcg)-50% (as indicated by manufacturer). Fish nuggets were prepared in three different formulations by incorporating corn starch (4%), green tea extract (0.1% (w/w of fish mince)) and BHT (0.01%). The composition of products were as follows: i) Fish mince+4% corn starch+1% salt – Control ii) Fish mince+4% corn starch+1% salt +0.1% green tea extract (GTE) iii) Fish mince+4% corn starch+1% salt + 0.01% BHT (BHT). Fish mince and ingredients were mixed well by using silent cutter (SCHARFEN, Germany) and kept under chilled condition (2– 3°C) for 30 min for uniform setting. Further, it was filled in a petridish (Diameter*height: 50*17 mm) to get desired shape and steam cooked with lid for 30 mins. The products were immediately kept under chilled condition and cooled. They were further packed in polypropylene pouches and stored in chilled condition (2°C). Samples were drawn at known intervals for biochemical, microbiological and sensory analysis.

Total phenolic content of green tea extract was determined as described by Singleton and Rossi (1965). DPPH radical scavenging activity was measured according to Shimada et al. (1992). Proximate composition of raw fish and fish nuggets were analysed as per AOAC (2012) method. pH of homogenized samples were determined using a calibrated glass electrode digital pH meter (Cyberscan 510; Eutech Instruments, Singapore). Total volatile base nitrogen (TVB-N) and Trimethylamine nitrogen (TMA-N) content was evaluated by the method followed by Conway (1950). Peroxide value was estimated as per AOAC (2012) method. Thiobarbituric acid value were determined according to Tarladgis method (1960 Color of fish nuggets were estimated using Hunter colourimeter (Hunter lab, Reston, VA, USA). Results were expressed as L*, a* and b* values. The texture of fish nuggets was analysed by texture analyser (Perton Instruments, Model-TVT, 6700, India). Antimicrobial property was determined as per CLSI (2012) standard well diffusion technique using Muller Hinton Agar (MHA). Aerobic plate count was estimated as per method. Sensory analysis of fish nuggets was evaluated according to Meilgaard et al., (1999). For sensory analysis, restructured products were deep fried for 3 min after battering and breading. The sensory attributes evaluated were appearance, color, flavour, texture, taste and over all acceptability. Overall acceptability score below 5 was considered as level of rejection. All the data obtained were subjected to one way ANOVA (analysis of variances) using (SPSS Statistics 16.0) software program. Differences in the mean values of the various treatments were determined at 5% level of significance ($p<0.05$) using Duncan test.

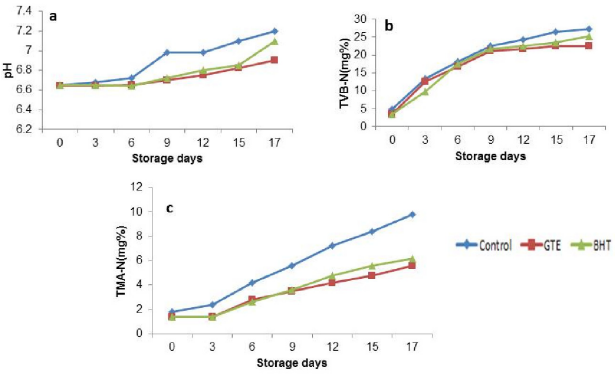

Fig. 1. Changes in a) pH b) TVB-N c) TMA-N content in fish nugget during chilled storage

Results and Discussion

The antioxidant effect of green tea extract are mainly due to the presence of polyphenols, predominantly the catechin (Abdel-Rahman et al., 2011). Total phenolic content of green tea extract used in the

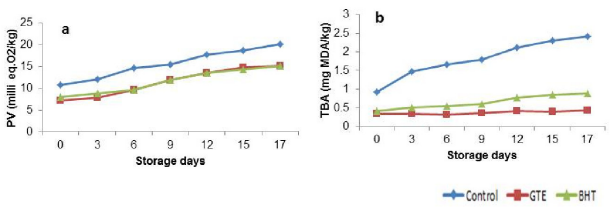

Fig. 2. Changes in a) PV b) TBA values in fish nugget during chilled storage

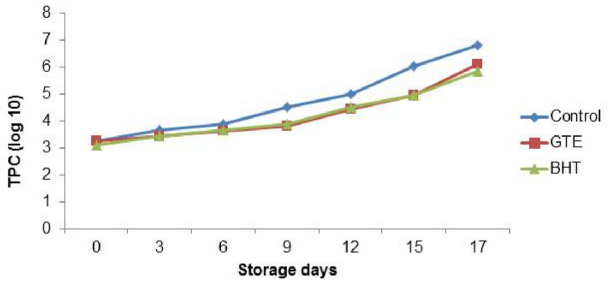

Fig. 3. Changes in TPC in fish nugget during chilled storage

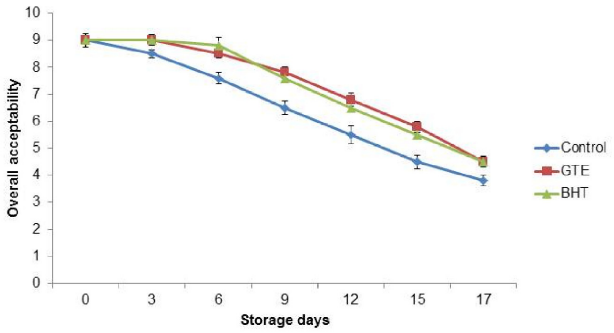

Fig. 4. Changes in overall acceptability of fish nugget during chilled storage

present study was found to be 191.75 mgl^{-1}. The relationship between presence of phenolic content and antioxidant effect of green tea extract has been extensively studied (Harborne & Williams, 2000; Heim et al., 2002). DPPH assay is one of the most commonly used method to evaluate antioxidant capacity of foods. DPPH radical scavenging activity of green tea extract (100 ppm) was 80.99±1.3%. Results are in agreement with previous reports for green tea extract (Singh etal. 2018; Horzig et al., 2009). Antimicrobial activity of green tea extract was tested against major food borne pathogen and spoilage microorganism viz., *Vibrio cholerae, V. parahaemolyticus, Staphlococcus aureus, Listeria monocytogens, Salmonella typhi, E. coli, Bacillus cereus, pseudomonas, Brochothrix thermosphacta, Lactobacillus* and H_2S Forming bacteria. Among the tested microorganisms, *B. thermosphacta* and *Pseudomonas species* indicated around 9 mm and 11 mm zone of inhibition, respectively. However, other bacterial population were found to be resistant to green tea extract at the tested concentration of 10 ppm. Parvathy et al. (2018) observed similar results for green coffee extract at the concentration of 50 ppm.

Pink perch (*Nemipterus japanicus*) mince had 81.46±0.25% moisture, 17.44± 0.20% protein, 0.85±0.05% fat and 1.02±0.02% ash. The present results were agreement with similar reports for fresh pink perch fish muscle (Jeyakumari et al. 2006; Murthy etal., 2017). Fish nugget had 75.65±0.35% moisture, 17.92±0.15% protein, 1.2±0.04% fat and 1.96±0.02% ash. In the present study, moisture content of fish nugget showed an increasing trend during chilled storage. Jeyakumari et al. (2016) observed similar results for restructured product prepared from pangasius surimi. Initial pH of the mince was 6.65±0.01. Fish nuggets had a pH range between 6.65±0.01 and 6.67±0.02. There was no significant difference in pH of fish nugget up to 6^{th} day. Thereafter it showed a gradual increase during storage (Fig.1a). Moreover, GTE and BHT had a lower pH than control. The increase in pH during storage in fish nugget might be due to the production of volatile bases by enzymatic and microbial activity on protein and other compounds (Jeyakumari et al. (2016). Initial total volatile nitrogen content of fish nugget was 4.9±0.02 mg% in control and it was increased to 27.3±0.03 mg% by the end of storage period. Fish nugget containing GTE and BHT had a TVB-N value of 22.4±0.01 mg%, 25.2±0.03 mg%, respectively at the end of storage period (Fig.1b). Results indicated that incorporation of GTE reduced the formation of total volatile base in the sample. Jeyakumari et al. (2017) reported that reduction in TVB-N content might be due to antimicrobial effect of plant extract which resulted in decreased microbial activity and formation of volatile nitrogen bases. Initial TMA-N content of fish nugget was 1.8±0.01 mg% in control and it was increased to 9.80±0.03 mg% towards the end of storage period. Fish nuggets containing GTE as well as BHT incorporated one, had a TMA-N value of 5.6±0.01 mg% and 6.2±0.04 mg%, respectively at the end of storage period (Fig.1c). Peroxide value of fish nuggets showed an increasing trend during storage. In control, peroxide value reached 18.84±0.20 mg% at the end of storage. However, GTE had a lower

Table 1. Changes in texture of fish nugget during chilled storage

Days	Sample	Hardnesss (N)	Chewiness (kgf.mm)	Springiness (mm)	Cohesiveness
0	Control	26.06 ± 1.4	8.42 ± 0.54	0.83 ± 0.01	0.40 ± 0.01
	GTE	25.3 ± 0.68	8.63 ± 0.19	0.89 ± 0.02	0.38 ± 0.01
	BHT	24.02 ± 0.36	8.36 ± 0.07	0.86 ± 0.01	0.40 ± 0.001
3	Control	30.95 ± 0.41	10.88 ± 0.20	0.88 ± 0.01	0.4 ± 0.01
	GTE	29.88 ± 0.36	10.84 ± 0.12	0.90 ± 0.01	0.40 ± 0.01
	BHT	28.75 ± 1.39	10.36 ± 0.59	0.89 ± 0.01	0.41 ± 0.01
6	Control	30.75 ± 2.3	11.25 ± 0.89	0.88 ±.01	0.42 ± 0.01
	GTE	33.84 ± 1.52	12.12 ± 0.53	0.88 ± 0.01	0.40 ± 0.01
	BHT	30.72 ± 0.66	11.85 ± 0.89	0.94 ± 0.06	0.42 ± 0.01
9	Control	33.85 ± 0.36	12.25 ± 0.55	0.86 ± 0.01	0.41 ± 0.01
	GTE	37.23 ± 1.52	12.95 ± 0.81	0.88 ± 0.01	0.4 ± 0.01
	BHT	31.94 ± 1.32	11.05 ± 0.56	0.84 ± 0.01	0.41 ± 0.01
12	Control	30.05 ± 0.25	12.09 ± 0.05	0.87 ± 0.01	0.41 ± 0.01
	GTE	37.02 ± 1.9	13.68 ± 0.52	0.9 ± 0.01	0.4 ± 0.01
	BHT	34.33 ± 0.30	12.65 ± 0.19	0.9 ± 0.01	0.41 ± 0.001
15	Control	39.19 ± 0.34	14.16 ± 0.26	0.89 ± 0.02	0.4 ± 0.01
	GTE	39.5 ± 0.66	14.99 ± 0.17	0.91 ± 0.01	0.41 ± 0.001
	BHT	40.11 ± 2.58	15.22 ± 0.01	0.89 ± 0.01	0.4 ± 0.01
17	Control	36.83 ± 0.44	13.30 ± 0.16	0.89 ± 0.02	0.4 ± 0.01
	GTE	43.18 ± 0.76	15.85 ± 0.25	0.9 ± 0.01	0.41 ± 0.01
	BHT	35.55 ± 2.14	13.43 ± 0.34	0.94 ± 0.06	0.4 ± 0.01

PV of 15.2±0.35 meq.O_2 kg^{-1} and it was comparable with BHT (15.12±0.30 meq.O_2 kg^{-1}) (Fig. 2a). Similar to PV, Thiobarbituric acid value also showed an increased trend during storage. Control sample crossed the limit of acceptability on 14th day. However GTE, BHT incorporated fish nuggets had a TBA value within acceptable limit throughout the storage period (Fig.2b). Parvathy et al. (2018) observed lower lipid oxidation in mackerel mince treated with green coffee extract and reported that incorporation of green coffee extract could inhibit the lipid oxidation. Tang et al. (2001) reported addition of tea catechins could inhibit lipid oxidation in cooked beef patties.

Texture analysis of fish nugget indicated that all the sample showed an increasing trend in hardness and chewiness values during storage. Horita et al. (2011) observed increase in the hardness in emulsified product and reported that it might be due to the reduction of bound water in the batter during cooking. Jeyakumari et al. (2016) observed similar results for restructured product prepared from pangasius surimi. Moreover, it was observed that up to 9th day there was a significant difference ($p<0.05$) in hardness value in GTE than control (Table 1). It might be due to the polymerisation of protein molecules with phenolic substances i.e more interactions or cross links restricting the flexibility of the protein aggregates resulting in the gels to become less springy and more rigid (Ranendra et al. 2017). Afterwards, no significant change was noticed between the samples during storage. It might be due to that products stored under lower temperature which resulted lower protein denaturation in the products. Ranendra et al. (2017) observed similar results for ginger extract incorporated fish sausage. Springiness and cohesiveness does not showed significant change throughout the storage period. This indicated that there was not much change in the internal bonding of cooked fish product.

Color analysis revealed a significant difference in L^*, a^*, b^*values of control and GTE. L^* value showed a decreasing trend in GTE during chilled storage. Parvathy et al. (2018) observed similar results for

Table 2. Changes in color value of fish nugget during chilled storage

Days	Sample	*L**	*a**	*b**
0	Control	62.62 ± 0.08	1.67±0.04	18.66±0.02
	GTE	59.29 ± .13	3.11±0.02	14.87±0.15
	BHT	63.06 ± .10	1.37±0.02	19.55±0.06
3	Control	63.76 ± 0.16	0.64±0.13	18.21±0.01
	GTE	57.6 ± 0.07	2.74±0.04	14.56±0.03
	BHT	63.04 ± 0.15	0.59±0.04	18.71±0.08
6	Control	64.24 ± 0.06	-0.24±0.02	17.74±0.01
	GTE	56.83 ± 0.19	3.14±0.08	15.26±0.06
	BHT	64.3 ± 0.09	0.08±0.03	18.08±0.15
9	Control	64.8 ± 0.03	-0.44±0.05	18.09±0.05
	GTE	55.97 ± 0.12	3.43±0.04	15.41±0.15
	BHT	63.58 ± 0.04	0.18±0.05	18.37±0.20
12	Control	64.41 ± 0.09	-0.32±0.05	17.8±0.05
	GTE	56.95 ± 0.17	3.1±0.03	15.5±0.04
	BHT	64.8 ± 0.11	-0.38±0.02	17.97±0.10
15	Control	65.39 ± 0.39	-0.11±0.02	18.81±0.04
	GTE	56.23 ± 0.03	4.16±0.01	15.9±0.06
	BHT	65.14 ± 0.07	-0.2±0.01	18.65±0.01
17	Control	67.05 ± 0.02	-0.39±0.01	18.77±0.15
	GTE	56.06 ± 0.01	4.44±0.01	15.21±0.20
	BHT	65.98 ± 0.04	0.15±0.01	19.28±0.15

fish mince treated with green coffee extract. However, control and BHT did not indicate any significant changes during storage period (Table 2). a* and *b** values showed an increasing trend in GTE during chilled storage. Moreover, a* and *b** values were found to be higher in GTE than control and BHT. This might be due to presence of green tea extract in fish nugget. Mitsuru et al. (2005) observed similar result for cooked beef patties. Although the colour changes in fish or fish product are mostly interrelated with lipid or protein oxidation, in the present investigation color changes in fish nugget was difficult to interpret, as the green tea extracts itself had a colour (greenish color) and itwas reflected in the incorporated product . An et al. (2004) reported that color changes cannot be stabilized by addition of antioxidant in raw beef patties during storage.

Total plate count showed an increasing trend during storage in all samples and the rate of increase was found to be comparatively lower in GTE and BHT incorporated sample. A 5 log cfu g^{-1} is recommended as permissible microbial limit for restructured products (Gilbert et al. 2000). Accordingly, TPC crossed 5log cfu g^{-1} for control on 15th day (Fig. 3). In case of GTE and BHT, it crossed the limit on 17th day. Organoleptic evaluation of fish nuggets showed significant ($p<0.05$) decrease in overall acceptability after 6th day for control and were rejected on 15th day. In GTE and BHT samples, a significant ($p<0.05$) decrease in overall acceptability was noticed after 9th day and both samples were rejected on 17th day (Fig. 4). Generally, changes in biochemical indices are well associate with the sensory score. However, in the present study, the decrease in overall acceptability score was well related to the microbial load during storage. Jeyakumari et al. (2016) observed similar results for restructured product prepared from pangasius surimi.

From the present study, it was concluded that both green tea extract incorporated nuggets as well as BHT added nuggets had a shelf life of up to 15 days under chilled storage (2^{o}C) than control (12days). Results suggested an incorporation of 0.1% green tea extract in fish nuggets and similar fish mince based

products for better stability as these natural extracts could serve as a potential natural antioxidant in foods.

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