ASSESSMENT OF THE SHELF LIFE OF FISH FILLET TREATED WITH GREEN TEA EXTRACTS UNDER REFRIGERATED CONDITION

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ABSTRACT: Value addition in fish processing industry is gaining importance because of the increased realization of foreign exchange and high unit value of such products including fillets. Due to the presence of PUFA fish lipids are highly susceptible to lipid degradation and addition of natural antioxidants with antimicrobial properties such as tea is one of the strategies to reduce or retard oxidation and prevent the loss of quality and sensory attributes. In the present study, the first and second extracts of green and black tea (Camelia sinensis) were used as an antioxidant and antimicrobial agent for Pangasiussutchi fillets. Phenolic compounds of tea extract resulted in microbial inhibition, protecting fillets against the internal protease and finally inhibit protein breakdown and amine production. Under refrigerated storage lower (p<0.05) TBARS, PV and FFA values were recorded in all treated samples and were within the limit of acceptability till 15 days indicating a significant antioxidant effect of 1st and 2nd extracts. Higher values of protein solubility (p<0.05) and WHC (p<0.05) in treatments concluded the preservative properties of tea extract that has successfully reduced the freeze induced protein denaturation. The lower values of TPC encountered in fillet treated with tea extract (p<0.05) suggests that both the green tea extracts possess antimicrobial properties that resulted in growth inhibition of the bacteria. The shelf life of the fillet treated with tea extracts is adjudged 12 days for refrigerated storage.

Key words: Green tea, Pangasius sutchi fillet, refrigerated storage, antioxidant.

INTRODUCTION

Addition of synthetic or natural antioxidant is one of the strategies to reduce or retard oxidation and prevent the loss of quality and sensory attributes of food (Serdarglu and Felekoglu, 2005). However, recent day consumers are showing less interest in purchasing products containing artificial additives. Again, addition of synthetic antioxidants has been restricted because of their health risks and toxicity (Linderschmidt et al, 1986). Therefore, attention has been diverted towards the potential of herbs and spices as antioxidative additives in food products leading to novel combinations of antioxidants and the development of novel food products (Perumalla et al, 2012). There is a growing interest in the use of natural plants because of their considerable role as functional and biochemical inhibitors of oxidative damage induced by free radicals.

Many plant tissues are good sources of phytochemicals, notably phenolic and flavonoids (Gorinstein et al, 2005) that can act as the best alternatives to synthetic food additives. Recently, plant phenolics have got an increasing interest in the food industry because they can retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Wojdylo et al, 2007). Among the natural plant extracts young leaves of Camellia sinensis has gained preference. It has been reported that the antioxidant activity of tea is comparable to synthetic antioxidants and hence can be used instead of synthetic antioxidants, which are potentially harmful (Cao et al, 1996). Tea is particularly rich in polyphenols, including catechins, theaflavins and thearubigins, which are thought to contribute to the health benefits of tea. Apart from antioxidant activity tea polyphenols are known for their antimicrobial properties.

Green tea has long been acclaimed for their antioxidative effects upon various foodstuffs and is known to contain substances that possess strong antibacterial activities which correspond to the presence of polyphenol compounds (Sakanaka et al, 2000). In green tea, major antibacterial polyphenol compounds are the catechin groups (Ooshima et al, 1993). Green tea is known to have the highest antioxidant capacity compared to other teas (Vinson, 2000) and has greater antioxidant effect than vitamin C, vitamin E, BHA (Butylated hydroxyanisole) and BHT (Butylated hydroxytoluene).
In this research, green tea extracts acquired from the leaves of *Camellia sinensis* were used as natural preservatives to preserve the *Pangasius* fish fillet under refrigerated storage. The purpose of this study is to evaluate green tea extract as a dipping medium for *Pangasius* fillet and to determine its effect on quality during refrigerated storage. The novelty of the work is the attempt made to reuse the tea leaves after first extraction as an antimicrobial or antioxidant agent. Reuse of tea leaves is expected to contribute to the lowering of the cost as well as put the otherwise waste tea leaves into a better use.

**METHODOLOGY**

Green tea leaves of standard variety free of additives were purchased from local market and the same variety was used throughout the experiment. Ten gram of ground dry green tea was added to 100 ml of distilled water and heated at 30-40°C for 45 min with a magnetic stirrer (DELA Model HM-101, Industries LTD) as described by Sarah *et al* (2010). The mixture was then filtrated with a Wattman filtration paper No.42 and the filtered solution with soluble solid content was applied as green tea extract (TE) in the experiment. After first extraction, the used leaves are again added to 100 ml distilled water and by following the same procedure a second extract of green tea is prepared. Preparation of the tea extracts was performed fresh prior to treatment in the laboratory.

Fillets were cut from freshly caught *Pangasius* fish. The fillets were tumbled in prepared dipping solution in a ratio 1:1 (w/v) for 10 minutes which is determined based on color, weight gain and sensory evaluation. Another group of untreated fillets were used as control. Before packaging with NY/LDPE laminated film bag, the fillets were placed carefully on a grid for 15 min to guarantee that the tea extracts effectively spread inside the muscle before packaging and to drain off excess solution liquid (Akse *et al*, 2008). Samples were placed in Styrofoam trays and stored at refrigerated condition (4±1°C) for up to 15 days.

The fillets were subjected in triplicate for Total Plate Count (TPC), physicochemical and sensory analyses at 3 days’ interval starting from day 0. TPC was determined according to standard American Public Health Association method (APHA, 2001) and results expressed as log CFU/g. The physicochemical indices used to analyse the shelf life of fish fillet were TVB-N (by the method described by Nath *et al*, 2014), PV (by the method described by Kirk & Sawyer, 1991), FFA (by the method as recommended by Nambudiri, 1985), Thio-Barbituric Acid Reactive Substances or TBARS (by EZAssay™ TM BARBS Estimation kit of Hi- Media Cell Culture following the method as described in the package insert), pH (by the method described by Benjakul *et al*, 1997, using a pH meter, Metrohm, 713ph Meter-Herisau Switzerland), protein solubility (by the method described by Lee *et al*, 1992 with some modification) and water holding capacity (WHC was measured by centrifugation as described by Shaviklo *et al*, 2010). Sensory evaluation was performed during storage as described by Nath *et al* (2016).

All of the data were checked for normal distributions with normality plots prior to analysis of variance (ANOVA), to determine significant differences among means at α = 0.05 level, using statistical tools of R software (ver. 2.14.1).

**RESULTS AND DISCUSSION**

The maximum permissible TPC for fresh water fish is 10^7 CFU/g as recommended by International Commission on Microbiological Specification for Foods. The changes in TPC of *Pangasius* fillet showed a gradual increase in all treatments from an initial value of 3.34±0.03 log CFU/g to 7.70±0.37 log CFU/g, 6.88±0.37 log CFU/g and 6.86±0.36 log CFU/g, for control, GT1 and GT2 respectively over a period of 15 days of storage under refrigerated condition (4±1°C) (Table 1) suggesting a significant (p <0.05) higher TPC in control sample than the treated samples. Both the treatments crossed the limit of acceptance of TPC after 12 days whereas to that of control samples was day 9. The lower values of TPC encountered in fillet treated with tea extract (p<0.05) suggests that both the green tea extracts possess antimicrobial properties that resulted in growth inhibition of the bacteria. Further, it is worth mentioning that the antimicrobial activity is not affected by the subsequent extraction process from used tea leaves. Similar findings were reported by Nurmahani *et al* (2012) that the antibacterial activity of PP & GT plant extracts may be due to the capability of bioactive compounds to form a complex with extracellular and soluble proteins, inhibit enzyme activity and also affect bacterial cell walls. Fan *et al* (2008) also attributed the lower TPC values to the antibacterial and antifungal impacts of TP.

TVBN serve as an indicator for the assessment of the freshness of fish. The total chemical compounds of; trimethylamine or TMA (produced by spoilage bacteria), dimethylamine or DMA (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites), and other volatile basic nitrogenous compounds associated with seafood spoilage are measured in TVB-N test (Huss,
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The TVB-N value ranges between of 30–35 mg/100 g for fish is generally regarded as the limit of acceptability for ice stored coldwater fish (Castro et al., 2006). Harpaz et al. (2003) reported that a level of 30 mg N/100 g is considered to be the upper limit, above which fishery products are considered unfit for human consumption. In the present study, the TVB-N value ranges between of 30–35 mg/100 g is considered to be the upper limit, above which fishery products are considered unfit for human consumption.

In the present study, the TVB-N value reaches to 31.74±1.8 mg %, 23.45±1.7 mg % and 26.34±1.6 mg % for control, GT1 and GT2 samples respectively (Fig. 1) from an initial value of 8.36±0.8 mg % over a period of 15 days of storage (p<0.05) under refrigerated condition (4±1°C). A significantly lower TVBN value (p<0.05) were recorded in all treated samples as compared to control till the end of storage suggesting a significant influence (p<0.05) of tea extract as an antimicrobial agent. The findings are in agreement with Nugraha et al. (2012) where fish without treatment had higher TVB – N level compared to green tea and black tea dipped sample. Although, the values of TVBN in treated samples were within the limit of acceptability (30–35 mg/100 g) till 15 days of storage under refrigerated condition, the increasing trend of TVB-N was may be due to enzymes, which are still active at low rates at cold temperatures, cause increase in the TVB-N values of the fish. Shinde et al. (2015) also reported similar findings where the initial TVB-N values (7.12±0.58 mg %) significantly increased (p < 0.05) without crossing the limit of acceptability in PPE and GTE treated samples during the chilled storage.

The peroxide value (PV), typically quantifying the primary lipid oxidation of products particularly the hydroperoxides (Chaijan, 2011) has been the most commonly employed chemical assay for evaluating oxidative stability of fats and oils. Peroxides are unstable compounds and they break down to aldehydes, ketones and alcohols, which are volatile and cause off-flavor in food products. The formation of FF A is a result of the enzymatic decomposition, particularly lipases and phospholipases, of lipid in frozen fish (Ucak et al., 2011) and independent of the storage temperature (Aidos et al., 2003). The acceptable limit for FFA is 15 mg/g (as oleic acid). Here, the initial FFA was 0.61±0.08% oleic acid, which finally reached to 2.02±0.13%, 0.61±0.14% and 0.64±0.11% oleic acid for control, GT1 and GT2 samples respectively (Fig. 3) over a period of 15 days of storage under refrigerated condition (4±1°C) suggesting all the samples were within the limit of acceptability. There is a significant (p<0.05) decrease in FFA values for treated samples as compared to control sample indicating a significant influence of green tea extract as antioxidant in decelerating the FFA production during storage. Similarly, Sarah et al. (2010) reported a lowered FFA content observed in 2.5% TE, 5% TE and 5% OJ (2.13%, 2.75% and 2.31%, respectively) compared to control after 8 days refrigerated storage.

The level of tissue malonaldehyde, a secondary degradation product of lipid present, is often measured as TBA in order to assess the extent of lipid peroxidation that has occurred in biological systems (Ucak et al., 2011). A TBA value in the range 1–2 mg malonaldehyde/kg of fish sample is usually taken as the limit of acceptability (Lakshmanan, 2000). Poor quality fish and fishery products have TBA value greater than 2.7 mg malonaldehyde /Kg leads to rancid smell and taste (Bonnell, 1994). In the present study, the TBARS value reaches to 2.27±0.04 mg MDA/kg, 1.04±0.04 mg MDA/kg and 1.16±0.03 mg MDA/kg for control, GT1 and GT2 samples respectively (Fig. 4) from an initial value of
0.14±0.01 mg MDA/kg over a period of 15 days of storage (p<0.05) under refrigerated condition (4±1°C). The increase in TBA value during the storage may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids.

The pH value assessed as a crucial factor for determination of meat quality (Nam et al., 2001), might interfere with solubility activities of antioxidants by changing in their electrical charges (Decker et al., 2005). In the present study, a gradual increase in the pH value of fish fillets was observed during 15 days of storage (p<0.05) under refrigerated condition (4±1°C). After 15 days, the pH value increased from initial 6.29±0.02 to 6.97±0.02, 6.34±0.07 and 6.42±0.05 for control, GT1 and GT2 samples respectively (Fig. 5) from which it can be stated that the highest increase in pH was observed in control sample. Application of green tea extract lowered the pH rise in the treated sample as compared to control.
sample which may be due to an increase in volatile bases compounds produced by either endogenous or microbial enzymes (Cann et al, 1983) and decomposition of nitrogenous components (Benjakul et al, 2002). It has been emphasized that the pH value can be raised to pH 7 or 8 during storage period. Additionally, Sikorski et al (1990) reported that the enzymatic degradation of ATP caused the liberation of inorganic phosphate and ammonia, leading to the changes in pH value.

Fig. 6 shows the decreasing trend in protein solubility of Pangasius fillet from an initial value of 86.19±1.45%, finally reaching to 63.08±3.5%, 65.48±3.5% and 65.25±3.45% for control, GT1 and GT2 samples respectively over a period of 15 days of storage under refrigerated condition (4±1°C), although the changes were non-significant (p>0.05).

Water holding capacity in meat tissue is strongly related to myofibril proteins. Reduction of water holding capacity occurs due to denaturation/aggregation of actin and in particular myosin, typically caused by ice crystal growth, increased ionic strength due to water crystallization and protein and lipid oxidation (Khidir et al, 2013), that finally leads to reduction of flavour agents and nutritive value (Reddy and Srikar, 1991). The internal factors influencing the water holding capacity of muscle tissue are species, age, size, muscle type, amount of intra muscular fat and muscle tissue condition post mortem, whereas the external factors are feeding patterns, season and location of catching and handling post slaughter (Khidir et al, 2013). In general, pH is commonly known to be one of the most important factors to affect the WHC of a product.Here, the initial WHC was 98.74±1.10%, which finally decreased to 73.28±2.65%, 79.6±2.63% and 77.95±2.62% for control, GT1 and GT2 samples respectively (Fig. 7) over a period of 15 days of storage under refrigerated condition (4±1°C) suggesting a significant (p<0.05) decrease in WHC values for control samples as compared to treated samples, thus, preservative properties of tea extract have successfully reduced the freeze induced protein denaturation.

The results of sensory evaluation (Figs. 8, 9, 10, 11) revealed that green tea extract treated samples stored under refrigerated condition (4±1°C) crossed acceptable limit on day 12 being rated “dislike slightly” whereas to
that of control samples was on day 9, by the panellists based on the color, texture, flavour and overall acceptability score.

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REFERENCES


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