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On

Green Tea Extracts as Natural Preservatives in Fish and Fishery Products

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Green Tea Extracts as Natural Preservatives in Fish and Fishery Products[1]

By

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ABSTRACT

The significant nutritional value of fish and fishery products, which include low-cost superior-quality proteins, vital fatty acids, and macro- and micronutrients, has attracted more attention. Due to quick microbial contamination that occurs naturally in fish, fish and fishery products are very perishable. To ensure quality and safety while extending shelf life, fish storage facilities frequently use synthetic preservatives. Because of their toxicity and cancer-causing qualities, synthetic antioxidants are hazardous to one's health. The persistent use of synthetic antioxidants, however, has been associated with several illnesses, including cancer. The food industry is now looking for natural preservatives due to consumer preferences for natural preservatives. As a natural and secure alternative to synthetic preservatives, researchers have been researching a variety of plant extracts. Studies has shown that adding green tea extracts to numerous food products can raise their quality and lengthen their shelf life. Green tea includes polyphenolic compounds having antioxidant and antibacterial effects that reduce oxidative rancidity in fish and fishery products. The bacterial count was significantly retarded, the sensory scores were significantly improved and the increase in total aerobic plate counts, pH, total volatile basic nitrogen and thiobarbituric acid reactive substances values were significantly inhibited in the fish and fishery products treated with green tea extracts than the control ones. And all these findings prove the efficient use of green tea extracts as natural preservatives in fish and fishery products for extending shelf life of fish and fishery products.

Keywords: Extracts, fishery products, green tea, preservatives

[1]Title of the seminar paper presented as a part of course FIT 598 during Winter 2022

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CHAPTER I

INTRODUCTION

Fish and fishery products (FFPs) are referred to all seawater or freshwater animals, whether wild or farmed, including all edible forms, parts, and products of such animals like filleted fish, smoked fish, cooked fish or canned fish (European Commission, 2004). Edible fishery products are fresh, chilled, frozen, salted, smoked and dried fish, crustaceans and mollusks. FFPs are considered a valuable part of human nutrition due to their high level of polyunsaturated fatty acids (PUFAs) and protein (Ozogul *et al.,* 2006; Kykkidou *et al.*, 2009); long-chain polyunsaturated fatty acids in these products have attracted attention due to their protection of human cardiovascular illnesses (Ozogul *et al.*, 2006). These products have a limited shelf life because fish's flavor, taste, texture, scent, and shelf life can all be impacted by the oxidation of unsaturated fatty acids due to the abundance of free amino acids, volatile nitrogen bases, and higher final pH that restrict the product's shelf life (Mexis *et al*., 2009). Although extremely nutritive, FFPs are prone to deterioration if kept at room temperature. Fish is therefore treated using a variety of techniques such as icing, freezing, canning, fermentation, smoking, the application of preservatives and antioxidants. Chemical preservatives and synthetic antioxidants are employed to increase shelf life. A number of synthetic or chemical preservatives, including sodium benzoates, sodium nitrite, butylated hydroxy anisole (BHA), and butylated hydroxytoluene (BHT), among others, have demonstrated promise in preventing texture and color changes, unpleasant taste and odor, and the loss of nutrients in fish during storage (Olatunde & Benjakul, 2018). But synthetic antioxidants have been linked to toxicity, cancer risk, and other health issues. As a result, the use of synthetic antioxidants is strictly controlled. People have become increasingly interested in natural products free of chemicals in recent years (D. P. Xu *et al*., 2017). Because of this, consumers who are concerned about their health are demanding natural resources as alternatives to preserve fish in good condition for extended periods of time. Since that many organic molecules have antioxidant and antibacterial capabilities, using natural preservatives to lengthen the shelf life of fish products is a promising prospect (Negro *et al*., 2003).

Recently, natural preservatives for fishery products have received a lot of attention, and new natural preservatives are constantly being found. The most typical sources of these natural compounds are microorganisms, animals, and plants (Olatunde & Benjakul, 2018). These organic additives offer a variety of antibacterial and antioxidant qualities that keep fish fresh and increase shelf life

(Gokoglu, 2019). For the preservation of food, numerous plant extracts have been utilized (Pazos *et al*., 2006; Khan *et al.*, 2009).

The antioxidant activity of green tea, which is derived from the leaf of the *Camellia sinensis* plant, has been compared to that of synthetic antioxidants, which have the potential to be harmful. Green tea is one of the most popular beverages in the world due to its pleasant flavor and use as a drug with therapeutic effects (Amira *et al*., 2010; Cao *et al*., 1996). Several studies have documented how green tea extract can prevent unsaturated fatty acid oxidation in a variety of seafood preparations (Lin & Lin, 2005; Nirmal & Benjakul, 2011).

Green tea also has a variety of pharmacological properties, including those that are anticarcinogenic, antiangiogenic, antioxidative, hypocholesterolemia, ant-obesity, anti-inflammatory, anti-atherosclerotic, antimutagenic, antibacterial, antiviral, antidiabetic, and anti-aging (Çelik, 2006). The ability of tea catechins to act as antioxidants and scavenge free radicals is their most important characteristic. Tea catechins may be utilized to lessen oxidation for mackerel patties while being stored in a chilled (4°C) and illuminated environment (Tang *et al.*, 2001). According to certain research, green tea prevents Parkinson's disease and also fights colon cancer (Koo & Cho, 2004) and enhances renal function (Mowafy *et al*., 2011). These characteristics place green tea extracts in the category of safe food additives.

This review provides an update about the effectiveness of green tea extracts (GTE) in preserving the quality and extending shelf-life of FFPs, with notes on their antimicrobial and antioxidant activities which will help to identify the need of novel natural seafood preservatives that is not only important for seafood preservation but also public health protection.

Objectives of the Study

The specific objectives of this review paper are as follows:

i. To evaluate the importance of using GTE as natural preservatives in FFPs
ii. To find out the effect of using GTE on shelf life and quality of FFPs

CHAPTER II

MATERIALS AND METHODS

This paper is entirely a review paper. So, this paper is mainly based on secondary information. Different published reports and articles are used to prepare this paper. Information has been assembled from various articles published in various journals, reports, publications and websites available on the online platform.

Constructive suggestions from my major professor and course instructors helped me to improve this paper. After the collection of all the related information, it was gathered and logically presented in the current form.

CHAPTER III

REVIEW OF FINDINGS

Since this paper is entirely a review, the key findings are presented here together with appropriate discussions in accordance with the initially stated objectives.

3.1 Green Tea Extracts

3.1.1 Composition

Polyphenols are the major components of tea leaves (Balentine *et al*., 1997). Caffeine (about 3.5% of the dry weight of the fresh tea leaves), theobromine (0.15-0.2%), theophylline (0.02-0.04%), and other methylxanthines, along with lignin (6.5%), organic acids (1.5%), chlorophyll (0.5%), and other pigments, theanine (4%), free amino acids (1-5.5%), and various flavoring compounds are all present in the fresh tea leaves (Graham, 1992). There are also a large range of other substances, such as flavones, phenolic acids and depsides, carbohydrates, alkaloids, minerals, vitamins, and enzymes (Chaturvedula & Prakash, 2011). Flavanols, primarily quercetin, kaempferol, myricetin, and their glycosides, are also present in tea. The green tea polyphenols, primarily the catechins, which account for 25–35% of the dry weight of green tea leaves, are responsible for most of the benefits of drinking green tea (Abdel-Rahman *et al*., 2011).

Due to variations in how the tea leaves are processed after harvest, green tea has much more polyphenols than black or oolong tea. To prevent the oxidation of catechins and keep the polyphenols in their monomeric forms, the polyphenol oxidases are deactivated in green tea by steaming and drying freshly collected tea leaves at high temperatures. Green tea catechin extract that is highly concentrated and has a mild flavor can be created by the processes of separation, purification, concentration, and drying. Temperature and pH affect how stable green tea catechins are. Green tea catechins are exceptionally stable in acidic solutions (pH 4); nevertheless, they are quite unstable in alkaline solutions (Zhu *et al*., 1997). Due to oxidation, thermal degradation, epimerization, and polymerization during heat treatments, green tea catechins lose some of their antioxidant properties. The extraction of green tea, which contains a wealth of bioactive components, is thought to be a difficult undertaking due to the presence of numerous functional features that might change depending on the extraction method, solvent, and duration used (Ng *et al*., 2020).

3.1.2 Mode of Action of Green Tea Extracts as Antioxidant

A significant challenge for seafood producers is the lipid oxidation and development of rancidity which shortens the shelf life of their products and changes their quality and nutritional content. The most frequent process causing the oxidative degradation of dietary lipids is known as autoxidation, which is a free radical chain reaction. The main byproducts of autoxidation have been identified as lipid hydroperoxides. Aldehydes, ketones, alcohols, hydrocarbons, and acids are produced during the breakdown of hydroperoxides, which are referred to as the secondary oxidation products of lipids. These substances are frequently to blame for the off flavors and odors in FFPs. The seafood sector is interested in antioxidants because they can halt the progression of oxidative rancidity in seafood (Abdel-Rahman *et al*., 2011).

The antioxidant effects of flavonoids, which make up most tea polyphenols, are well established. The chemical structure of green tea polyphenols, which includes aromatic rings and hydroxyl groups, is principally responsible for their antioxidant effect. These hydroxyl groups bind to and neutralize lipid free radicals. Numerous studies have shown that polyphenols and tea catechins are outstanding electron donors and efficient scavengers of physiologically relevant reactive oxygen species in vitro, such as superoxide anions (Nanjo *et al*., 1993; Guo *et al*., 1999; Nakagawa & Yokozawa, 2002; Michalak, 2006; Velayutham *et al.*, 2008), peroxyl radicals, and singlet oxygen (Guo *et al*., 1999; Michalak, 2006). By chelating redox active transition-metal ions, catechins also demonstrate antioxidant action.

Typically, transition metal ions can start free radical chain oxidations by releasing lipid alkoxyl radicals from the hemolytic breakage of the O-O link in lipid hydroperoxides (LOOH), which decomposes LOOH. By interacting with these lipid alkoxyl radicals, phenolic antioxidants, such as tea catechins, prevent lipid peroxidation. The quantity and position of hydroxyl groups in the molecules as well as their structure all affect this action (Milic *et al*., 1998). By preventing pro-oxidant enzymes and stimulating antioxidant enzymes, green tea catechins also demonstrate antioxidant action (Velayutham *et al*., 2008).

3.2 Application of Green Tea Extracts in Fish and Fishery Products

Green tea extracts already play an important role in increasing the shelf life of FFPs. Green tea extracts (GTE) have been proven to have antioxidant and antibacterial characteristics, which increase the shelf life of fish and shrimp products by preserving their quality (Firdous *et al*., 2020; Lin and Lin, 2005). Karsil *et al*., (2021) found that adding GTE improved the microbiological, physicochemical, textural, and sensory quality of rainbow trout fillets than the control one stored at 2±1°C.

Table 1. Some studies on applications of GTE to extend the shelf life of FFPs

Form of application	FFP	Important findings	Reference
Tea polyphenol	Japanese sea bass (*Lateolabrax japonicus*)	Inhibited total viable count in fish fillets treated with GTE under both frozen and chilled stored conditions than the untreated samples	Ju *et al*., (2017)
Tea polyphenol	Crucian carp (*Carassius auratus*)	Total viable count growth was delayed in the treated fish fillets by application of extracts	T. Li *et al*., (2012)
Ethanolic extract	Pacific white shrimps (*Litopenaeus vannamei*)	Lowered thiobarbituric acid reacting substances value in GTE treated shrimps suggesting oxidation inhibition	Nirmal & Benjakul, (2011a, 2011b)
Frozen GTE	Nile tilapia (*Oreochromis niloticus*)	Samples stored on frozen GTE had better biochemical and microbiological characteristics than the control	El *et al*., (2011)
Tea polyphenol	*Pangasius sutchi* fillets	Phenolic compounds of tea extract resulted in microbial inhibition, protecting fillets against the internal protease and finally inhibit protein breakdown	Pal *et al*., (2017)
Ethanolic extract	Pacific White Shrimp	Significantly decreased thiobarbituric acid reacting substances, total bacterial count suggesting oxidation and microbial inhibition in the GTE treated shrimps than the control	Hatab *et al*., (2018)

3.3 Effect of Green Tea Extracts in Fish and Fishery Products

3.3.1 Chemical Analysis

3.3.1.1 pH

The shelf life of Nile tilapia (*Oreochromis niloticus*) fish using frozen GTE with different concentrations (2, 4 and 6%, w/v) incresed during storage period at 4^0C and the pH levels of the control samples increased during frozen storage, reaching 6.83 after 6 days (El *et al.*, 2011). The enzymatic breakdown of the fish muscles and the formation of volatile basic components by spoilage bacteria may be to blame for this pH increase (Ruiz-Capillas and Moral, 2001). Table 2 further shows that there was only a minor increase in pH for GTE stored samples, with values of 6.70, 6.69, and 6.68 observed for samples placed on 2, 4, and 6% frozen GTE after 10, 14, and 14 days, respectively. It can be concluded that the lower pH of GTE-treated samples can improve microbial inhibition and prolong the preservation of fish samples for tea polyphenol's ability to suppress the activity of endogenous proteases. The pH rise in the treated sample was less than that in the control sample after the application of green tea extract. The pH of the mackerel fillets treated with GTE was also lower than the untreated samples when stored in ice (John *et al*., 2019).

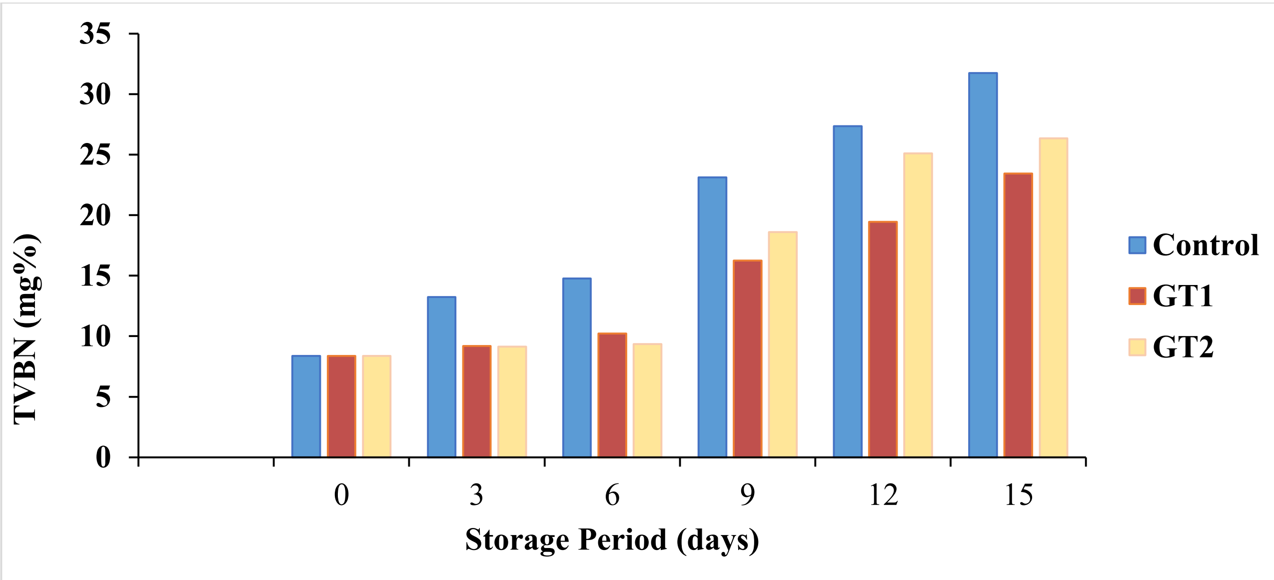
Table 2. Changes in pH values of tilapia fish as affected by iced storage on different concentrations of frozen green tea extract (GTE)

Storage period (days)	**Concentrations**			
	Control	**2% GTE**	**4% GTE**	**6% GTE**
0	6.51	6.50	6.50	6.49
2	6.63	6.52	6.51	6.50
4	6.70	6.55	6.52	6.51
6	6.83	6.59	6.54	6.52
8	-	6.62	6.55	6.53
10	-	6.70	6.58	6.56
12	-	-	6.63	6.59
14	-	-	6.69	6.68

Source: (El *et al.*, 2011)

3.3.1.2 Total Volatile Base Nitrogen (TVB-N)

An indicator for determining how fresh a fish is TVB-N. TVB-N is a byproduct of the decomposition of protein and non-protein nitrogen molecules brought on by microbial activity and endogenous enzymatic action, and it primarily consists of ammonia, trimethylamine, and dimethylamine (Huss, 1995). According to Harpaz *et al*., (2003), the top limit at which fishery products are regarded unfit for human consumption is 30 mg N/100 g.



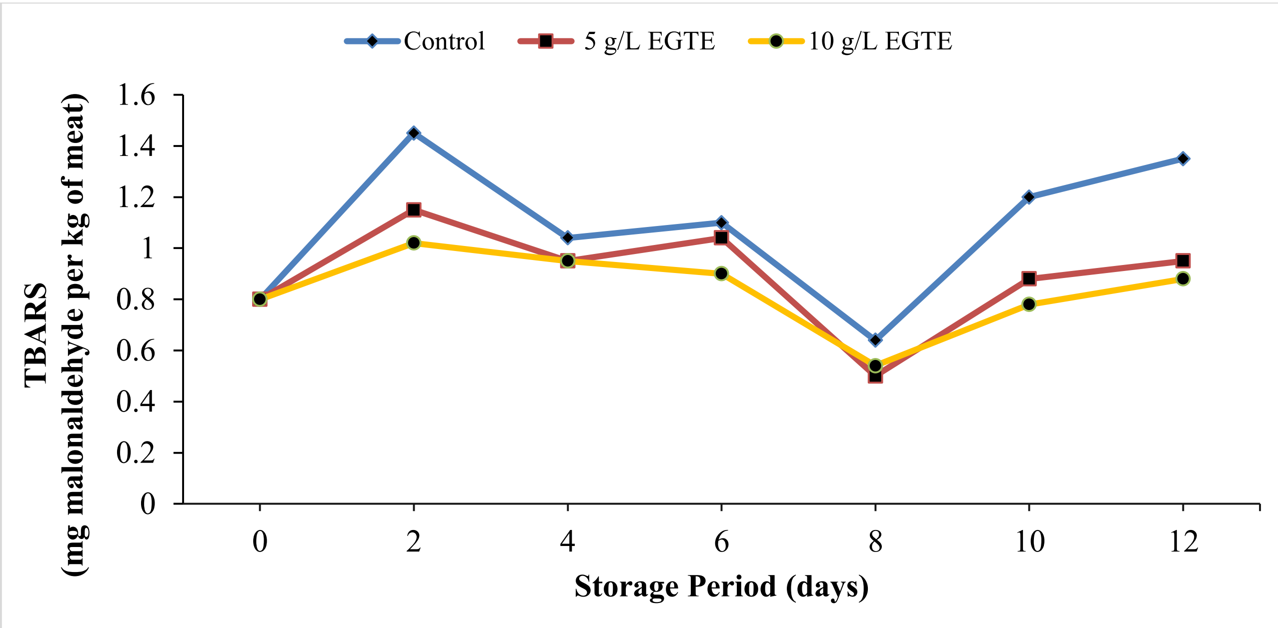
Source: (Pal *et al.*, 2017)

Figure 1. Changes in TVBN of *Pangasius* fillet treated with green tea extract under refrigerated condition (4±1°C).

According to Pal *et al*., (2017), shelf life of *Pangasius* fillet treated with green tea extracts of distilled water extraction at concentrations of 10g/100ml was extended under refrigerated condition where the TVB-N value reaches to 31.74 mg %, 23.45 mg % and 26.34 mg % for control, GT1 and GT2 samples respectively (Figure 1) from an initial value of 8.36 mg % over a period of 15 days of storage under refrigerated condition (4±1^{o}C). Up to the end of storage, all treated samples had TVB-N values that were considerably lower than controls, indicating that tea extract had a considerable antioxidant effect. The results were in agreement those of Nugraha *et al*., (2012) who found that fish not treated had greater TVB-N levels than samples dipped in green tea. However, the TVBN levels in the treated samples remained within the acceptable range (30 mg/100 g) after 15 days of storage in a refrigerator.

3.3.1.3 Thiobarbituric Acid Reactive Substances (TBARS)

To determine how much lipid peroxidation has happened in biological systems, tissue malonaldehyde, a secondary lipid breakdown product, is frequently quantified as TBA (Ucak *et al.*, 2011). The general limit of acceptance for a TBA value for a fish sample is between 1-2 mg malonaldehyde/kg (Lakshmanan, 2000). Rancid taste and odor are caused by low-quality fish and fishery products with TBA values greater than 2.7 mg malonaldehyde/kg (Bonnell, 1994).



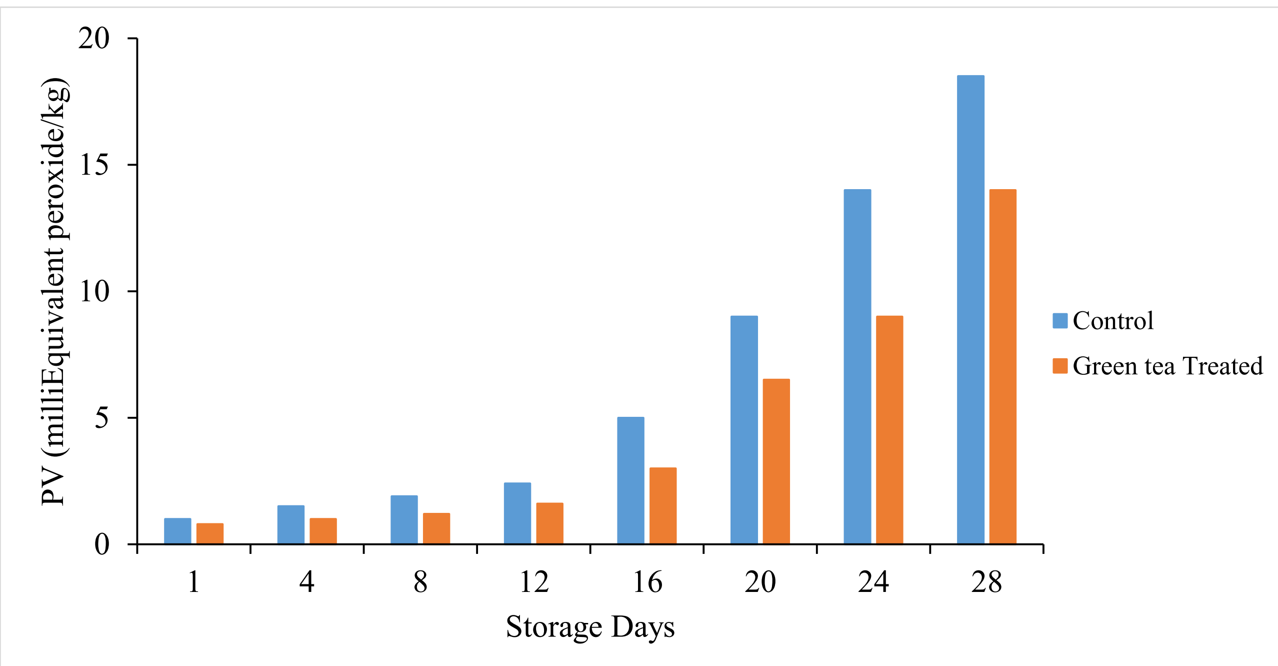
Source: Nirmal *et al.*, (2011a)

Figure 2. Thiobarbituric acid reactive substances of Pacific white shrimp treated with ethanolic green tea extract at different levels during 12 days of iced storage. EGTE: Ethanolic Green Tea Extract.

Effect of using ethanolic GTE at concentrations of 5 and 10 gL^{-1} for inhibition of polyphenoloxidase and retardation of quality loss of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage was evaluated by Nirmal *et al.*, (2011a). The results demonstrated that TBARS levels in treated fish generally increased within the first two days. Following that, the continual decline was noticed from days 4 through 8. Yet, the significant rise was apparent from days 8 to 12. In comparison to shrimp treated with ethanolic green tea extract during ice storage for 12 days, a higher TBARS value was seen in the control group. Shrimp treated with 10 gL^{-1} of ethanolic green tea extract had the lowest TBARS values at day 12 of storage, followed by shrimp treated with 5 gL^{-1} of extract. The 'leaching out' impact of those secondary products by melting ice during iced storage is most likely what caused the decline in TBARS value during 2–8 days of storage. Overall, lipid oxidation was reduced in shrimp treated with green tea extract at both concentrations when compared to the control, particularly after 8 days of storage. The results agreed those of Hassan & Geethalakshmi, (2020) who found that Japanese threadfin bream (*Nemipterus japonicus)* not treated had greater TBARS value than samples incorporated with GTE in chilled storage.

3.3.1.4 Peroxide Value (PV)

The most widely used chemical assay for assessing the oxidative stability of fats and oils is the peroxide value (PV), which typically quantifies the primary lipid oxidation of products, particularly the hydroperoxides (Chaijan, 2011). Lajolina *et al*., (1983) determined that the PV up to 30 meq of O_2/Kg lipids is regarded as acceptable in the absence of any undesirable off-taste or odor. Firdous *et al.*, (2020) assessed the effect of ethanol extracts of green tea (*Camellia sinensis* L.) on quality of chilled stored Indian white prawn *(Fenneropenaeus indicus)* for 28 days of storage period. Biochemical (total volatile nitrogen, free fatty acid and peroxide values), bacteriological (aerobic counts) inhibition of chilled stored shrimp was addressed to determine the efficacy of extracts as preservative.



Source: Firdous *et al.*, (2020)

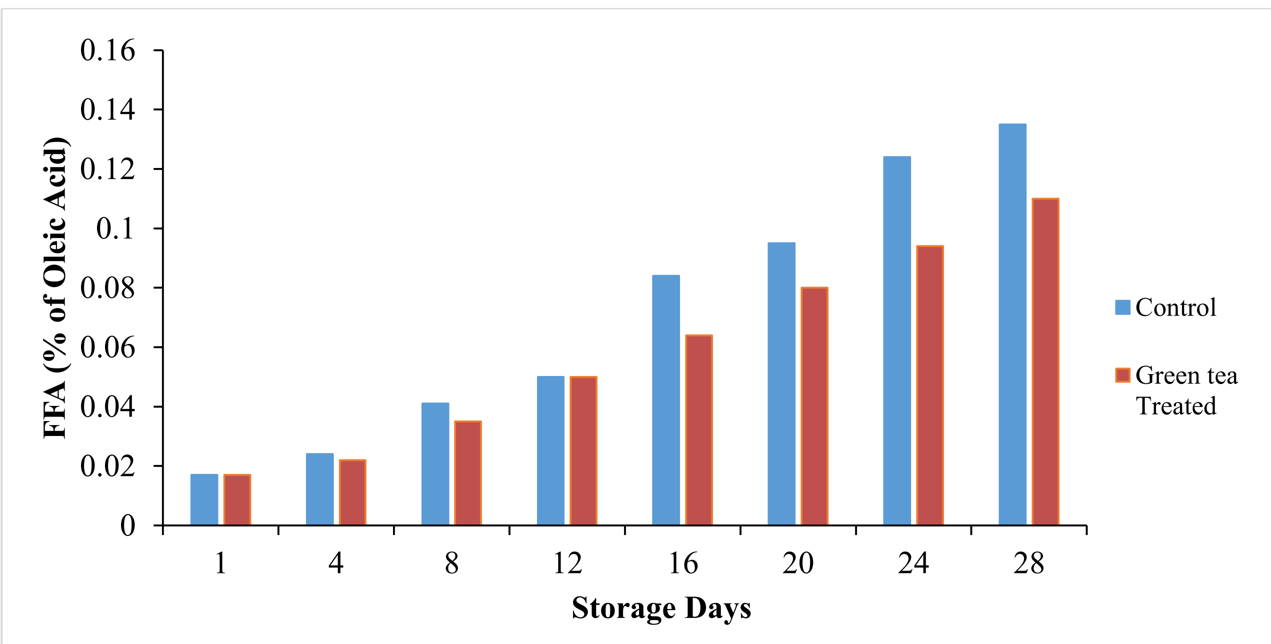
Figure 3. Peroxide value (PV) of Indian white prawn treated with green tea extract and control during 28 days of storage at 4^{o}C.

From the figure 3, we can see that PV increased from 0.81 milliequivalents/kg to 18.46 milliequivalents/kg in the control group, while green tea samples revealed a significantly lower level of 13.82 milliequivalents/kg from the initial value of 0.66 milliequivalents/kg. These PV values were significant different between Indian white prawns treated with green tea and control. In a similar trend of PV values, Haghparast *et al*., (2011) observed that green tea-treated Persian sturgeon during refrigerated storage showed a lower rise in PV compared to control. A combination of a glazing treatment and the application of green tea extract at a 5% concentration revealed a decrease in PV in the GTE treated bonito fillet compared to the control sample in Lin and Lin's (2005) study on the effect of glazes of various tea extracts upon the storage stability of bonito fillet.

3.3.1.5 Free Fatty Acid (FFA)

Fish oil damage during storage can be evaluated using the free fatty acid production as a result of increasing oxidation and enzymatic hydrolysis of unsaturated fatty acids (Srikar and Hiremath, 1972), and it can be used as a quality indicator for fish and other food products. FFA are created when the lipid in frozen fish is broken down by enzymes, mainly lipases and phospholipases (Ucak *et al.*, 2011). FFA is allowed up to a maximum of 15 mg/g (as oleic acid).

According to Firdous *et al.*, (2020), the effect of ethanol extracts of green tea (*Camellia sinensis* L.) on quality of chilled stored Indian white prawn *(Fenneropenaeus indicus)* showed promising effects on biochemical parameters followed by enhanced sensory attributes.



Source: Firdous *et al.*, (2020)

Figure 4. Free fatty acid content of Indian white prawn treated with green tea extract and control during 28 days of storage at 4^{o}C.

In control sample, increased the FFA value from 0.017% to 0.137%, whereas values of 0.017% to

0.112% were revealed for green tea treated sample after 28 days of storage (Figure 4) and it was observed a lower lipid hydrolysis rate in treated slots irrespective of their antioxidant contents. This finding is in accordance with that revealed by Sarah *et al*., (2010) where refrigerated Persian sturgeon (*Acipenser persicus)* treated with green tea lower the FFA content compared to the untreated group after 8 days of storage.

Table 3. Use of GTE as antioxidant for the preservation of FFPs

FFP	Concentration of GTE	Chemical quality indices	Reduction in final amount	References
Bonito fillet	5% v/w	PV	Yes +	Lin and Lin,
		TBARS	Yes +	(2005)
Kilka	200 ppm	PV	Yes +	Ojagh *et al*.,
		TBARS	Yes +	(2005)
Mackeral fillet	2000 ppm	PV	Yes ++	John *et al.,*
		TVB-N	Yes ++	(2019)
Marinated	2% w/v	FFA	Yes ++	Bilgin *et al*.,
anchovies		TVB-N	Yes +	(2018)
Pacific White	1% w/v	TBARS	Yes ++	Hatab *et al*.,
Shrimp				(2018)

The following classification has been used: ++ high reduction compared to control; + medium compared to control; PV – peroxide value; TVB-N – total volatile base nitrogen; TBARS – thiobarbituric acid reactive substances; FFA – free fatty acid

3.3.2 Sensory Analysis

Employment of frozen GTE at various concentrations (2, 4 and 6%, w/v) and its effect to extend the shelf life of Nile tilapia (*Oreochromis niloticus*) fish was assessed by El *et al*., (2011). Panelists evaluated the control and treated samples' sensory qualities as well as their general acceptability. A scale used to assess sensory quality that was created using the recommendations made by Lima dos Santos *et al.,* (1981). The scores were given on a scale from 10 to 9, with 9 being exceptional, 8 being good, and 6 being fair and acceptable. The panel's mean score served as a measure of the panel's overall sensory quality (Huss, 1995). A fish is discarded if it receives a score below 4.

Table 4. Scale employed for sensory evaluation of tilapia (*Oreochromis niloticus)* during iced storage

Score	Odor	Texture	General appearance
10	Fresh sea-weedy odor	Very firm, elastic to finger touch, muscle not yet in rigor	Very fresh, shiny appearance, reddish white meat
9	Loss of fresh sea-weedy odor	Moderately firm, elastic, muscle in pre-rigor	Fresh, shiny, meat in reddish white in color
8	No odors, neutral odor	Firm, moderately elastic, muscle in rigor	Fresh, slight loss in shiny appearance
7	Slightly musty, mousy, garlic odor	Fresh Slightly firm, slightly elastic, muscle in rigor	Slight loss in freshness, slight change in reddish white meat color
6	Bready, malty, yeasty odor	Slight soft, muscle passing out of rigor	Loss in freshness, pale white meat
5	Lactic acid, sour milk odor	Moderately soft, muscle in postrigor	Meat color in light white, slightly bleached
4	Butyric or acetic acid, or chloroform odor	Soft, slightly loose flesh	Complete loss in freshness, meat in milky white color, slightly bleached
3	Stale cabbage water, phosphine-like odor	Very soft, loosened flesh	Completely bleached, meat yellowish white in color
2	Ammoniacal odor	Very soft and flabby, slight retaining of finger indentation, flesh easily torn	Discolored, pale yellowish meat
1	Fecal, H_2S, strong ammoniacal and putrid odors	Extremely soft and flabby, strong retention of marks, flesh very easily torn	Completely discolored, yellowish meat

Source: Lima dos Santos *et al.*, (1981)

Table 5 lists the sensory evaluations of the tilapia fish kept on frozen GTE. All of the samples at zero time were in great condition, with ratings ranging from 9.5 to 9.6 and being handled in a fresh manner. The panelists rejected all of the samples after 6, 10, 14 and 14 days for the control sample and those stored on frozen GTE with concentrations of 2, 4, and 6%, respectively, due to a significant deterioration in fish quality over the storage period.

Table 5. Changes in the sensory characteristics of tilapia fish as affected by iced storage on different concentrations of frozen GTE

Storage period (days)	**Concentrations**			
	Control	**2% GTE**	**4% GTE**	**6% GTE**
0	9.6	9.50	9.51	9.50
2	7.3	8.92	9.30	9.33
4	5.1	8.00	9.00	9.00
6	3.5	6.21	8.23	8.34
8	-	5.10	7.88	7.90
10	-	3.89	7.00	6.95
12	-	-	5.93	5.89
14	-	-	4.00	4.00

Source: (El *et al.*, 2011)

We can infer from the statistics that GTE treated samples may be maintaining their high-quality attributes in terms of sensory evaluation.

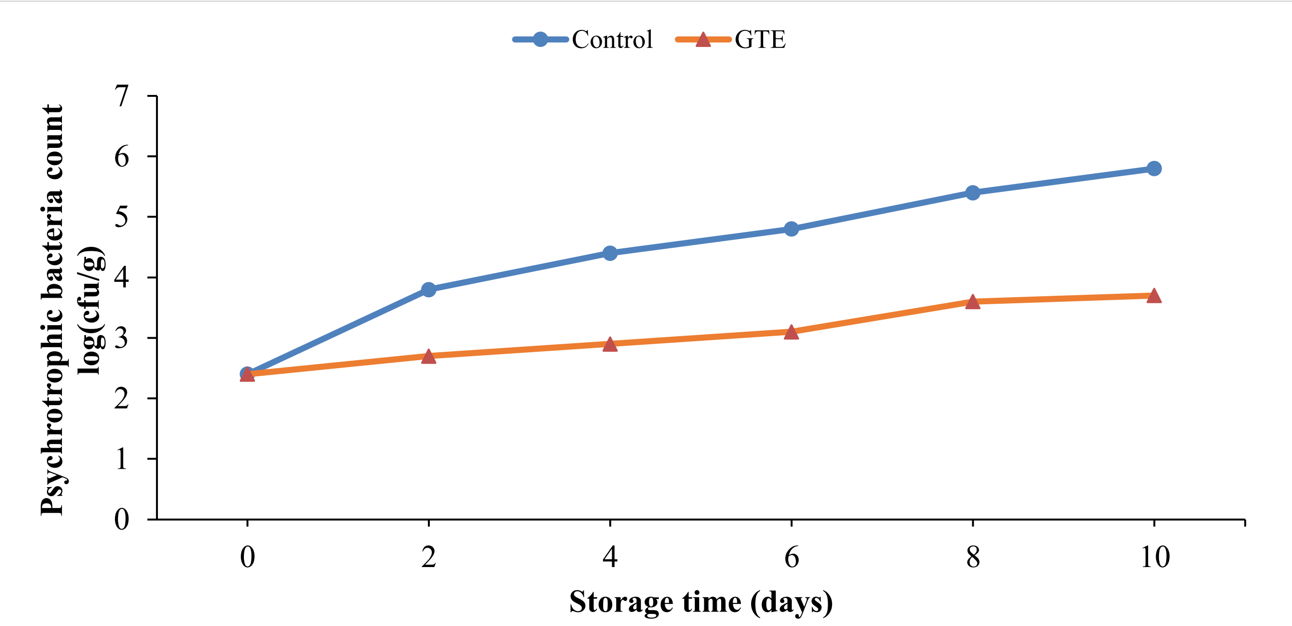
Table 6. Overview of studies about effect of GTE on the organoleptic properties of FFPs

FFP	**Concentration of GTE**	**Organoleptic aspects**	**Effect on the organoleptic properties**	**References**
Pangasius fillet	10% w/v	Color	+	Pal *et al.*, (2017)
Mackeral fillet	2000 ppm	Color	+	
		Odor	+	John *et al*., (2019)
		Taste	+	
Rainbow Trout Fillets	10% v/w	Appearance	+	Karsli *et al.*, (2021)
		Color	++	
		Odor	+	
Pacific white shrimp	10% w/v	Color	+	Nirmal & Benjakul, (2011)
		Odor	+	

The following classification has been used: ++ high reduction compared to control; + medium reduction compared to control

3.3.3 Microbial Analysis

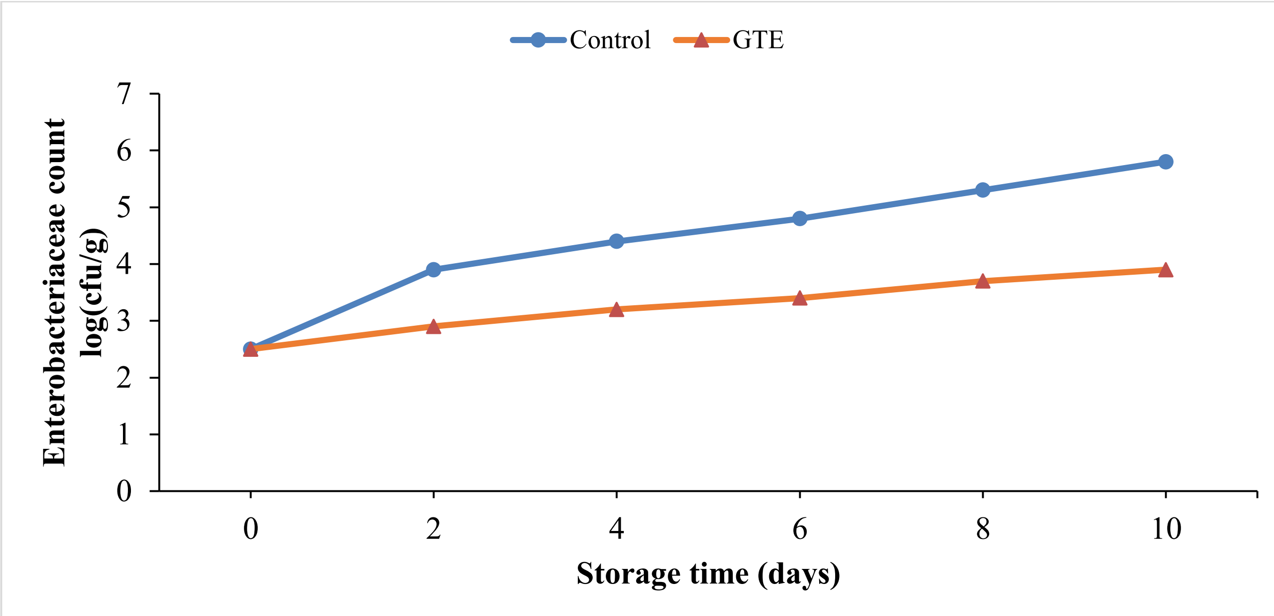
The treatment of Pacific white shrimp (*Litopenaeus vannamei*) with green tea extract (1g/L; GTE) during refrigerated storage of 10 days had an impact on the quality alterations of the shrimp (Nirmal *et al.*, 2011b). Microbiological investigations included counts of enterobacteriaceae, psychrotrophic bacteria, and bacteria that produce H_2S.



Source: Nirmal *et al.*, (2011b)

Figure 5. Psychrotrophic bacteria count of Pacific white shrimp without and with GTE treatment during 10 days of storage at 4°C.

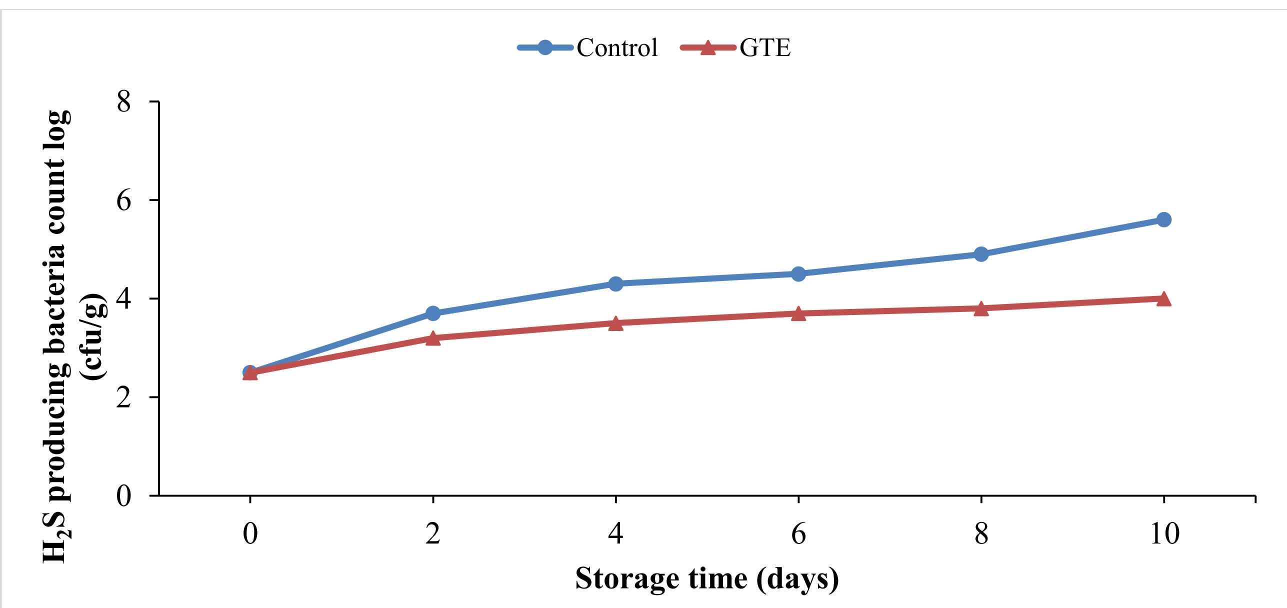
All samples had a psychrotrophic bacteria count (PBC) of 2.4 log cfu/g on the first day of storage (Figure 5). In general, as storage time increased, PBC of the control (stored in air) increased steadily. Hence, treatment of shrimp with GTE more effectively slowed the growth of psychrotrophic bacteria than control. This finding was in agreement with that of El *et al*., (2011) who found that tilapia (*Oreochromis niloticus*) fish treated with 4% GTE treatment was effective in preventing the growth of psychotrophic bacteria and extending the storage time of fish samples in ice to 14 days as opposed to 6 days for control treatment.



Source: Nirmal *et al.*, (2011b)

Figure 6. Enterobacteriaceae count of Pacific white shrimp without and with GTE treatment during 10 days of storage at 4°C.

According to Nirmal *et al.*, (2011b), at day 0 of storage, 2.5 log cfu/g of Enterobacteriaceae were identified in all samples (Figure 6). During their 10-days period of refrigeration, shrimp treated with GTE slowed enterobacteriaceae growth more efficiently than controls.



Source: Nirmal *et al.*, (2011b)

Figure 7. H_2S producing bacteria count of Pacific white shrimp without and with GTE treatment during 10 days of storage at 4°C.

For all samples, the number of H_2S-producing bacteria in shrimp at day 0 was 2.5 log cfu/g (Figure 7). All samples indicated a general increase in the number of bacteria that produce H_2S over time, but the control sample increased at a higher rate. The results agreed with those of Hassan & Geethalakshmi, (2020) who found that Japanese threadfin bream (*Nemipterus japonicus)* not treated had greater counts of H_2S producing bacteria than samples incorporated with GTE in chilled storage.

In order to determine the shelf life of *Pangasius* fillet, green tea extracts were utilized as an antibacterial agent (Pal *et al*., 2017). The phenolic components in tea extract inhibited the growth of microbes.

The fillets underwent total plate count (TPC). TPC was calculated using the American Public Health Association's recommended methodology, with the results reported as log cfu/g. The International Committee on Microbiological Specification for Foods recommends a maximum allowable TPC of 10^7CFU/g for freshwater fish.

Table 7. Changes in TPC of *Pangasius* fillet treated with green tea extract under refrigerated condition (4±1°C)

Days	C	GT1	GT2
0	3.34	3.34	3.34
3	3.54	3.40	3.41
6	4.58	4.16	4.17
9	5.33	4.28	4.85
12	6.60	5.65	5.52
15	7.70	6.88	6.86

Source: Pal *et al*., (2017)

After 15 days of storage at 4±1°C, the TPC of Pangasius fillets increased gradually in all treatments from an initial value of 3.34log CFU/g to 7.70log CFU/g, 6.88log CFU/g, and 6.86log CFU/g, for the control, GT1, and GT2, respectively (Table 7). This suggests that the control sample had a significantly higher TPC than the treated samples. After 12 days, both treatments exceeded TPC's acceptance criteria, whereas the control samples did so on day 9.

The fact that fillet treated with tea extract had reduced TPC values implies that both green tea extracts have antibacterial characteristics that prevented bacteria from growing.

Table 8. Use of GTE as antimicrobial for the preservation of FFPs

FFP	Concentration of GTE	Bacterial species	Reduction in final population	References
Mackeral fillet	2000 ppm	TPC	Yes +	John *et al.*, (2019)
Rainbow Trout Fillets	10% v/w	TAMB PBC	Yes + Yes +	Karsli *et al.*, (2021)
Marinated anchovies	2% w/v	TVC	Yes +	Bilgin *et al.*, (2018)
Pacific White Shrimp	1% w/v	TPC	Yes ++	Hatab *et al.*, (2018)
Pacific white shrimp	10% w/v	PBC	Yes ++	Nirmal & Benjakul, (2011)

The following classification has been used: ++ high reduction compared to control; + medium reduction compared to control; TPC - total plate counts; TVC - Total viable count; TAMB - total aerobic mesophilic; PBC - psychrophilic bacteria count

Antimicrobial activities of green tea extracts for the preservations of FFPs are described in Table 8. According to the data of Table 8, it can be said that green tea extracts with different concentrations significantly retarded bacterial counts.

CHAPTER IV

CONCLUSIONS

Based on the findings of this review paper, the following conclusions are drawn.

i. While it is usual practice to employ synthetic antioxidants and antibacterial to maintain the quality of fish and fishery products, customer concern about their safety has prompted the seafood sector to look for natural alternatives. GTE contain polyphenolic compounds having the antioxidant and antimicrobial properties that inhibit oxidative rancidity in fish and fishery products. Polyphenols of GTE have beneficial anti-bacterial and anti-oxidative activities, which demonstrates potential for their use as the preservatives and the antioxidants in seafood industry especially in the field of the preservation. Hence, GTE extend the scope for serving many useful functions to fish processing sector.

ii. Nevertheless, the results of many research studies show that GTE as natural preservatives contribute to extend the shelf life and improve the quality of fish and fishery products. The bacterial count was significantly retarded, the sensory scores were significantly improved and the increase in total aerobic plate counts, pH, total volatile basic nitrogen and thiobarbituric acid reactive substances values were significantly inhibited in the fish and fishery products treated with GTE than the control ones. Hence, use of green tea extracts could be an effective alternative to synthetic preservatives and are highly recommended as natural preservatives to extend the shelf life of fish and fishery products.

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