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POTENTIAL OF GREEN TEA EXTRACTS COMBINED WITH ICE TO EXTEND THE SHELF LIFE OF NILE TILAPIA FISH

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ABSTRACT

The feasibility of using different concentrations of green tea extract (0; 2; 4 and 6%) in combination with ice to extend the shelf life of Nile tilapia *(O.niloticus)* fish was examined. Control and treated fish samples were analyzed periodically (every two days) for the biochemical {pH, total volatile basic nitrogen (TVBN); Trimethylamine nitrogen (TMA-N) and thiobarbituric acid (TBA)}; microbiological {total viable bacterial count (TVBC); psychrophilic bacteria; total mold and yeast count} and sensory characteristics during cold storage period at 4 ±1 °C.

The results indicated that the treated samples with green tea extract increased in shelf-life at cold storage which rejected at 10; 14 and 14 day of storage for samples treated with 2, 4 and 6% green tea extract in comparison with 6 days for the control. Thus, green tea extract in combination with ice can be used as a natural preservative and to prolong the shelf-life of Nile tilapia fish at cold storage at 4 ±1°C and the best concentration was 4% green tea extract because it was less expensive than the use of 6% green tea extract giving the same effect on the shelf life.

Keywords: Shelf life; Tilapia fish; Ice; Spoilage, Green tea

INTRODUCTION

The two major problems with respect to marketing and distribution of seafood are their high perishability and poor hygienic quality. This is essentially due to contamination of the commodity by spoilage and pathogenic microorganisms (Gram & Huss, 1996 and Garrett, *et al.*, 1997). The spoilage of fish is a complex process in which physical, chemical and microbiological mechanisms are implicated. Iced storage is, an efficient way of reducing the rate of the deterioration of fish and also of extending the shelf life of fish. Many fish species have been examined during storage on ice (Ryder, *et al.*, 1993; Namulema, *et al.*, 1999 and Karungi, *et al.*, 2004). However, the quality of fish muscle will also deteriorate during iced storage as the fish muscle is abundant in proteins and unsaturated fatty acid. Endogenous proteases, which are able to hydrolyze different proteins in the fish muscle, are

rtant early in the deterioration process (Cepeda, *et al.*,1990), so taking some sures to delay the decline of fish quality and extending the preservation life of during ice storage is worthwhile.

Tea polyphenols (TP), which play an important role in protein precipitation enzyme inhibition (Cartriona, *et al.*, 1988; Shi, *et al.*, 1994), have beneficial bacterial and anti-oxidative activities, which demonstrate potential for their use ie preservatives and the anti-oxidants in food industry especially in the field of preservation of manufactured meat.

The aim of the present study is to increase the shelf life of Nile tilapia *iloticus)* fish using different concentrations of green tea extracts (0; 2, 4 and in combination with ice at cold storage.

MATERIALS AND METHODS

paration of crushed ice combined with green tea extracts

Green tea (2, 4 and 6 gm/100 ml) was extracted for 1 min. in distilled water 0 °C at levels 2; 4 and 6%, then crushed ice was prepared from different tea act concentrations, control crushed ice was prepared from distilled water.

nple preparation:

Fresh Nile tilapia *(o.niloticus)* weighing 180 to 200g/fish were obtained from production ponds in Central Laboratory for Aquaculture Research. Abbassa, Abu-nmad; Sharkia, Egypt, and immediately transported to the Fish processing and ility control Lab. Fish were washed with cold water and were divided to four ches. The first batch was stored under crushed ice left as control and the other ches were stored under crushed ice with different levels (2, 4 and 6%) of green extracts with a fish: ice ratio 1:1. All samples were put in polystyrene boxes ch boxes contains a bottom layer of crushed ice and ice on the top which npletely covered the fish. An outlet at the bottom of the boxes facilitated removal water from the melting ice. The boxes were stored at 4±1°C, ice was replenished riodically. Fish samples were taken randomly every two days for analyzing for chemical, microbiological and sensory characteristics.

Determination of total phenolic:

Total phenolic content was analyzed according to the Folin-Ciocalteu method (Julken-Titto, 1985). One milliliter of Folin-Ciocalteu reagent (Merck Co., Darmstadt, and FRG) diluted with 2 ml of distilled water was added to 50 µl of tea extract. The color of mixture was then developed using sodium carbonate. The absorbance of derived product was read at 735 nm, using Gallic acid (Sigma Chemical Co.) as a standard for the calibration curve. The results were expressed as milligrams per gram of Gallic acid equivalents.

Biochemical index

The pH was assessed using a pH meter on a homogenate consisting of 5 g of fish fillets in 50 ml of distilled water as described by Carballo *et al.* (1995). The total volatile basic nitrogen and trimethyleamine were determined according to the method of AMC (1979). Thiobarbituric acid was determined according to the method described by Tarladgis *et al.,* (1960).

Microbiological analysis:

Total bacterial count (TBC); and Psychrophilic bacteria were determined as recommended by the APHA (1992) using plate count agar medium. Total mold and yeast counts were determined as described by Oxoid Manual, (1982) using Oxytetracycline glucose yeast extracts agar (Oxoid CM 545) medium.

Sensory analysis:

Sensory characteristics and overall acceptability of Nile tilapia fish *(O. Niloticus)* were assessed by a panel of six experienced panelists on the basis of a ten-point scale on each sampling. Sensory characteristics studied included general appearance, odor and texture of fish. Scale employed for evaluating sensory quality developed based on the guidelines given by Lima dos Santos *et al.,* (1981). Scale employed for evaluating sensory quality of chilled tilapia is given in Table (1). The scores were given in the decreasing order scale with 10–9 for excellent, 8–7 for good, 6–5 for fair and acceptable, 4–3 for poor and 2–1 for very poor. The mean of the scores given by the panel represented the overall sensory quality (Huss, 1995). A score less than 4 indicate that the fish was rejected.

Table (1) Scale employed for sensory evaluation of tilapia *(O. Niloticus)* stored in tea extract combined with ice at cold storage

Score	Odour	Texture	General appearance
10	Fresh seaweedy odour	Very firm, elastic to finger touch, muscle not yet in rigor	Very fresh, shiny appearance, reddish white meat
9	Loss of fresh seaweedy odour	Moderately firm, elastic, muscle in pre-rigor	Fresh, shiny, meat in reddish white in colour
8	No odours, neutral odour	Firm, moderately elastic, muscle in rigor	Fresh, slight loss in shiny appearance
7	Slightly musty, mousy, garlic odour	Slightly firm, slightly elastic, muscle in rigor	Slight loss in freshness, slight change in reddish white meat colour
6	Bready, malty, yeasty odour	Slight soft, muscle passing out of rigor	Loss in freshness, pale white meat
5	Lactic acid, sour milk odour	Moderately soft, muscle in post rigor	Meat colour in light white, slightly bleached
4	Butyric or acetic acid, or chloroform odour	Soft, slightly loose flesh	Complete loss in freshness, meat in milky white colour, slightly bleached
3	Stale cabbage water, phosphine-like odour	Very soft, loosened flesh	Completely bleached, meat yellowish white in colour
2	Ammoniacal odour	Very soft and flabby, slight retaining of finger indentation, flesh easily torn	Discoloured, pale yellowish meat
1	Faecal, H2S, strong ammoniacal and putrid odours	Extremely soft and flabby, strong retention of marks, flesh very easily torn	Completely discoloured, yellowish meat

Statistical analysis:

The obtained data were conducted to two-way ANOVA to test the effect of green tea extract levels and time intervals. The differences among means were significant at significance level of $P < 0.05$ using Tucky test as a post-hoc test. All statistics were run on the computer using the SAS program **(SAS, 2000).**

RESULTS AND DISCUSSION

The levels of total phenolic in the three tea extracts prepared from green tea was found to be 57.7; 115.4 and 170.2 mg/g. for 2; 4 and 6% green tea extracts.

The pH values in relation to the storage time are presented in Table (2). The initial pH values were 6.51, 6.50, 6.50 and 6.49 for control and samples treated with different concentration of green tea extract (2, 4 and 6%) respectively, during iced storage, the pH values of control samples were significantly increased reaching 6.83, at the 6 th day of storage. This increase in the pH may be due to the enzymatic degradation of the fish muscle. These results are in agreement with Amira Elhanafy (2005). From the same table it could also be seen that the pH of treated samples were non significantly increased up to the 6 the day of cold storage followed by significant increase slightly reached to 6.70, 6.69, and 6.68 for samples treated with 2, 4, and 6% green tea extract at the 10, 14 and 14 day of iced storage, respectively. These results may be due to Tea polyphenols (TP), which play an important role in protein precipitation and enzyme inhibition and have beneficial anti-bacterial and anti-oxidative activities, Cartriona, *et al.,* (1988); Shi, *et al.,* (1994).

Table (2) Effect of different concentrations of green tea extract combined with ice on the pH value of tilapia *(O. Niloticus)* fish during cold storage at 4 ± 1 °C.

Storage period (days)	Concentrations (treatments)			
	control	2%	4%	6%
0	6.51±0.02a	6.50±0.03a	6.50±0.05a	6.49±0.06a
2	6.63±0.03b	6.52±0.05a	6.51±0.05a	6.50±0.03a
4	6.70±0.03b	6.55±0.04a	6.52±0.07a	6.51±0.05a
6	6.83±0.02b$_{®}$	6.59±0.05a	6.54±0.06a	6.52±0.06a
8	--	6.62±0.01b	6.55±0.03b	6.53±0.06a
10	--	6.70±0.02c$_{®}$	6.58±0.04bc	6.56±0.07c
12		--	6.63±0.02b	6.59±0.03b
14		--	6.69±0.04b$_{®}$	6.68±0.04b$_{®}$

$_{®}$ Rejected

Total volatile basis Nitrogen (TVBN) is well documented as a good index of the quality of fresh or frozen fish because its increase is related to spoilage by the activity of endogenous enzymes and bacterial growth. El-Marrakchi, *et al.,* (1990) and Harpaz *et al.,* (2003) They reported that a level of 30 mgN/100g TVBN is

considered to be the upper limit, above which fishery products are considered unfit for human consumption.

There was a gradual significantly increment in the initial TVBN in ali samples during iced storage, differences in TVBN could be observed between control and treated samples in this experiment Table (3), The TVBN of the control samples reached 37.78 mg N/100g at the 6 th day of cold storage this means that these samples are considered unfit to human consumption according to Egyptian standards, (2005). Meanwhile, TVBN reached to 36.97, 35.90 and 34.81 mg N/100g at 10, 14 and 14 days of cold storage for tilapia fish treated with 2, 4 and 6% of green tea extract, respectively. This can be attributed to either a more rapidly reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both, which was due to the effect of total polyphenoilc in green tea (Banks, *et al.,* 1980).

It could be also noticed that the TVBN of samples treated with 4 and 6 % green tea extract were approximately the same at the end of the storage period.

Table (3) Effect of different concentrations of green tea extract combined with ice on the total volatile basic nitrogen (TVBN) of tilapia *(O. Niloticus)* fish during cold storage at 4 ± 1°C.

Storage period (days)	Concentrations (treatments)			
	control	2%	4%	6%
0	8.56±0.3a	8.55±0.06a	8.54±0.08a	8.54±0.3a
2	18.95±0.6c	12.92±0.1b	10.10±0.2a	9.31±0.4a
4	24.31±0.3c	18.12±0.2b	13.76±0.8a	12.77±0.1a
6	37.78±0.1c®	23.60±0.2b	17.93±1.07a	16.31±0.06a
8	--	29.28±0.7c	19.03±0.5b	19.00±0.07b
10	--	36.97±0.2c®	23.25±0.1b	22.88±0.3b
12		--	28.01±0.2b	27.75±0.4b
14		--	35.90±0.3c®	34.81±0.2b®

® Rejected

Low initial TMA-N content (approximately 0.83 mg N/100g. fish muscle) (Table 4) indicates that the tilapia fish were of good quality as reported by Egyptian standards (2005).During old storage period TMA-N production of 2; 4 and 6% green tea extract samples were significantly lower than for control samples which were 12.53; 10.93; 11.00 and 10.92 mg/100g sample at 10; 12; 14 and 14 days of cold

storage for the control; 2; 4 and 6% treated samples respectively. This can be attributed to either a more rapidly reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both because of the effect of total polyphenoilc in extracted green tea (Banks, *et al.,* 1980).

TBA measurements in Table (5) showed that there was a significant increase in lipid oxidation in both control and those treated with different concentrations of green tea extract. However, the increase rate of TBA values of treated fish was lower than the control throughout the period of cold storage. The TBA value of the control reached 3.99 mg malonaldehyde/kg at the 6 th day of cold storage, While TBA values of treated samples were 2.98, 1.72 and 1.67 mg malonaldehyde/kg for 2, 4 and 6% green tea extract treatments at the 10, 14 and 14 th days of cold storage respectively, these results may be attributed to Tea polyphenol which play an important role protein precipitation and enzyme inhibition (Shi, *et al.,* 1994 and Fan *et al.,* 2008), they reported that the tea extract have beneficial anti-bacterial and anti-oxidative activities, which demonstrates potential for their use as the preservatives and the anti-oxidants in food industry especially in the field of the preservation of manufactured meat.

Table (4) Effect of different concentrations of green tea extract combined with ice on the trimethylamine-nitrogen (TMA-N) value of tilapia *(O. Niloticus)* fish during cold storage at 4± 1°C.

Storage period (days)	Concentrations (treatments)			
	control	2%	4%	6%
0	0.83±0.1a	0.83±0.1a	0.82±0.07a	0.81±0.06a
2	2.99±0.2b	1.08±0.2a	0.98±0.05a	0.92±0.02a
4	7.82±0.04c	2.79±0.2b	1.42±0.2a	1.28±0.05a
6	12.53±0.3c®	6.82±0.04b	3.51±0.3a	2.89±0.1a
8	--	9.01±0.4c	4.93±0.4b	3.98±0.2b
10	--	10.93±0.3c®	7.21±0.3b	6.79±0.1b
12		--	9.30±0.1c	9.00±0.01b
14		--	11.00±0.2b®	10.92±0.6b®

® Rejected

Total bacterial count of control and treated samples was followed during cold storage. The initial total bacterial count of the control was 4.36 log cfu/g.

Storage at 4 °C ±1 considerably increased the total bacterial counts in control samples reached 7.89 log cfu/g at the 6 th day of cold storage Table (6). Kim *et al.,* (1995) stated that the shelf-life of fresh water fish fillets is strongly influenced by the initial microbial quality, it is generally agreed that spoilage of fresh water fish occurs within 5-8 days under refrigerated temperature.

Table (5) Effect of different concentrations of green tea extract combined with ice on the thiobarbituric acid (TBA) value of tilapia *(O. Niloticus)* fish during cold storage at 4 ± 1°C.

Storage period (days)	Concentrations (treatments)			
	control	2%	4%	6%
0	0.28±0.09a	0.27±0.04a	0.26±0.07a	0.27±0.03a
2	0.69±0.05a	0.43±0.1a	0.30±0.06a	0.29±0.06a
4	1.07±0.2ac	0.69±0.04b	0.38±0.06a	0.37±0.3a
6	3.99±0.1b®	1.08±0.04a	0.56±0.06a	0.48±0.3a
8	--	1.92±0.04c	0.77±0.06b	0.63±0.09b
10	--	02.98±0.05c®	0.92±0.03b	0.98±0.05b
12		--	1.02±0.06b	1.04±0.06b
14		--	1.72±0.08b®	1.67±0.09b®

® Rejected

Table (6) Effect of different concentrations of green tea extract combined with ice on the total viable bacterial count (TVBC) (log cfu/g) of tilapia *(O. Niloticus)* fish during cold storage at 4± 1 °C.

Storage period (days)	Concentrations (treatments)			
	control	2%	4%	6%
0	4.36±0.09a	4.34±0.05a	4.36±0.07a	4.32±0.03a
2	5.59±0.05b	4.83±0.1a	4.61±0.07a	4.59±0.06a
4	6.67±0.2b	5.08±0.04a	4.79±0.06a	4.77±0.3a
6	7.89±0.1c®	5.99±0.03b	5.27±0.08a	5.20±0.1a
8	--	6.69±0.04c	5.89±0.06b	5.85±0.09b
10	--	7.41±0.05c®	6.23±0.03b	6.17±0.05b
12		--	6.98±0.07b	6.95±0.06b
14		--	7.14±0.08b®	7.11±0.09b®

® Rejected

Treating tilapia fish with different concentrations of tea extract had no real effect on the initial total bacterial counts which were 4.34; 4.36 and 4.32 log cfu/g for 2; 4 and 6% green tea extract treatments at zero time respectively. Meanwhile, during cold storage period the total bacterial count for the control sample was significantly increased reaching 7.89 log cfu/g at the 6 th day of storage, and the treated tilapia fish were significantly increased during cold storage reaching 7.41,

7.14 and 7.11 log cfu/g at 10, 14 and 14 day of cold storage for green tea extract treated sample with 2, 4 and 6% respectively. These results could be attributed to the antibacterial effect of tea polyphenol according to Fan *et al.,* 2008. The maximum acceptable count for fresh water fish is 10^7 cfu/g as recommended by ICMSF (1978).

Treating Nile tilapia samples with different concentrations of green tea extract had no effect the initial total psychrophilic counts Table (7) which were around 3.71 log cfu/g for both control and samples treated with 2, 4 and 6% green tea extract respectively. Cold storage at 4 °C significantly increased the total psychrophilic count for both control and treated sample which reached to 6.69, 5.88, 5.11 and 5.07 log cfu/g. for control and treated sample with 2, 4 and 6% at the 6, 10, 14 and 14 day of cold storage respectively Fan *et al.*,(2008).

Cold storage caused a gradual increase in total mold and yeast counts reaching 3.53 log cfu/g at the 6 day of cold storage for the control where the panelists completely rejected this sample because of the visual appearance of mold spots on the surface of tilapia fish Table (8). Regarding to treated samples there was a slightly increase in the total mold and yeast counts during cold storage reaching 3.46, 3.23 and 3.07 log cfu/g for 2, 4 and 6% green tea extract treatment at the 10, 14 and 14 days of cold storage, respectively

Table (7) Effect of different concentrations of green tea extract combined with ice on the psychrophilic bacterial count (log cfu/g) of tilapia *(O. Niloticus)* fish during cold storage at 4± 1°C.

Storage period (days)	Concentrations (treatments)			
	control	2%	4%	6%
0	3.72±0.02a	3.71±0.01a	3.70±0.07a	3.70±0.09a
2	3.99±0.04a	3.85±0.09ab	3.72±0.07b	3.71±0.01b
4	4.93±0.05a	3.99±0.02b	3.78±0.04c	3.76±0.06c
6	6.69±0.03®	4.63±0.04	3.96±0.02	3.93±0.08
8	--	4.94±0.08a	4.07±0.03b	4.04±0.05b
10	--	5.88±0.07a®	4.67±0.1b	4.62±0.03b
12		--	4.94±0.05a	4.90±0.1a
14		--	5.11±0.08a®	5.07±1a®

® Rejected

Table (8) Effect of different concentrations of green tea extract combined with ice on the total mold and yeast count (log cfu/g) of tilapia *(O. Niloticus)* fish during cold storage at 4 ± 1°C.

Storage period (days)	Concentrations (treatments)			
	control	2%	4%	6%
0	1.07±0.02a	1.07±0.02a	1.11±0.07a	1.07±0.09a
2	2.27±0.04b	1.93±1ab	1.30±0.07a	1.14±0.01a
4	2.66±0.05c	2.25±0.02b	1.94±0.04a	1.85±0.06a
6	3.53±0.03c$_{®}$	2.86±0.04b	2.44±0.02a	2.11±0.08a
8	--	2.99±0.08c	2.78±0.03b	2.73±0.05b
10	--	3.46±0.07c$_{®}$	2.86±0.1b	2.84±0.03b
12		--	2.99±0.05b	2.94±0.1b
14		--	3.23±0.08b$_{®}$	3.07±1b$_{®}$

® Rejected

The sensory scores of the tilapia fish stored under different concentration of green tee extract combined with ice at cold storage are shown in table (9). All the samples at zero time were in fresh manner and had high scores ranged from 9.5 to 9.6 this means that all samples at zero time were in excellent quality according to (Huss, 1995), during the cold storage period there was a gradually significant loss in the fish quality for all samples which were rejected by the panelists at 6, 10, 14 and 14th for samples treated with 2, 4 and 6% green tea extract.

Conclusion

It can be concluded that green tea extract in combination with ice can be used as a natural preservative and to extend the shelf-life of Nile tilapia fish at cold storage at (4 ±1°C) and the best concentration was 4% green tea extract because it was less expensive than the use of 6% green tea extract giving the same effect on the shelf life.

Table (9) Effect of different concentrations of green tea extract combined with ice on the sensory characteristics of tilapia *(O. Niloticus)* fish during cold storage at 4 °C ± 1.

Storage period (days)	Concentrations (treatments)			
	control	2%	4%	6%
0	9.6±0.2b	9.50±0.03a	9.51±0.06b	9.50±0.04a
2	7.3±0.2a	8.92±0.08b	9.30±0.06c	9.33±0.06c
4	5.1±0.1a	8.00±0.1b	9.00±0.2c	9.00±0.1c
6	3.5$_{®}$±0.1a	6.21±0.06b	8.23±0.09c	8.34±0.09c
8		5.1±0.07a	7.88±0.1b	7.90±0.2b
10		3.89$_{®}$±1a	7.00±0.05b	6.95±0.03b
12			5.93$_{®}$±0.1a	5.89$_{®}$±0.2a
14			4.000.2a	4.00±0.2a

® Rejected

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