

Effect of Some Natural Extracts on Shelf Life of Chilled *Lucioperca Lucioperca* Fillets

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Abstract—The effects of green tea (GT), *Coriander sativum* (CS) and Parsley extracts were studied on *Lucioperca Lucioperca* after dipping for 10 minutes at a concentration of 0.3 % from different extracts. The organoleptic score acceptability of GT, CS and parsley treated groups were extended for 3, 6 and 15 days, respectively over control group. All extracts delayed increments of pH, total volatile basic nitrogen (TVB-N), and oxidative rancidity. Aerobic plate counts (APC), *Pseudomonas* and *Enterobacteriaceae* counts were greatly reduced in treated groups on comparison with control group during chilling days. Additionally, the formation of biogenic amines; Histamine, cadaverine and putrescine was reduced significantly during chilling period. Histamine level at the end of chilling period in control group (22.4 mg Kg⁻¹) exceeded the limit of Egyptian standard (20 mg Kg⁻¹) while, in all treated groups was below it.

Keywords—About four key words or phrases in alphabetical order, separated by commas.

I. INTRODUCTION

Fish is considered as important part of the Egyptian diet, and demand is expected to increase as we have shortage in production of red and white meats. Fish is richest sources of long-chain *n*-3 polyunsaturated fatty acids in nature. Despite their therapeutic and nutritional value, these products have a short shelf-life. This is due to the high amounts of free amino acids and volatile nitrogen bases and higher final pH that limit the useful life of the product being highly susceptible to oxidation of unsaturated fatty acids [1]. The quality of fish degrades under the complex process in which physical, chemical and microbiological forms of deterioration are happened [2].

Tea polyphenols (TP) are important bioactive materials extracted from (GT) which have health promoting properties likely related to the antioxidant abilities of phenolic contents [3],[4],[5]. The majority of polyphenolic compounds in green tea are catechins, that include epigallocatechin gallate, epigallocatechin, epicatechin gallate and epicatechin [6].

Coriander (*C. sativum* L.) belonging to the family *Apiaceae* is a glabrous aromatic, herbaceous annual plant, which has a long history as a culinary herb being the source of aroma compounds and EOs with biologically active components

possessing antibacterial, antifungal and antioxidant activities, and thus *C. sativum* is useful in food preparation [7]

Parsley is a plant that is used to add flavor to foods in china, Mexico and South America, India and Southeast Asia and have a good effect on taste. Free Phenolic acids or their ester and ether components that is found in all plant tissues are synthesized according a special pattern when plant confronts with environmental stress [8].

Lucioperca Lucioperca fresh water valuable fish is sold in Egypt after filleting and preserved by chilling. The objective of the current study is investigating the effect of some natural extracts on *Lucioperca Lucioperca* fillets kept chilled at 4 ±1°C.

II. MATERIALS AND METHODS

A. Sample Preparation

One hundred and twenty freshly prepared *Lucioperca Lucioperca* fish slices (70 ± 5g per slice) were collected from Al Obour city market, Qalyubia Governorate, Egypt.

Fish slices were divided into four groups first group (control) was stored after dipping in sterile distilled water, second group was dipped in 1% green tea extract, third group was dipped in 1% coriander sativum extract and fourth group was dipped in 1% parsley extract. All groups were kept dipped for 10 minutes then removed to a sterile steel mesh net in order to drip the excess dipping fluid. Each group was identified in large Ziploc bag and every slice was kept in sterile poly ethylene bag. All groups were kept in fridge at 4 ± 1°C and were examined at zero time and every three days interval.

B. Extract Preparation

Green tea extract: About 20 g of air dried and ground green tea leaves were mixed with 500ml of boiling water and left for 5 minutes. The extract was obtained by filtration and the soluble solid content of green tea extract was measured by using Abbe refractometer (Opton, Hamburg, Germany). Then we used 0.3% of green tea extract (6 ml dissolved in distilled water to 2 liters volume) as described by [9].

Coriander sativum: About 200 g of ground samples of coriander seeds were soaked in 500 ml of diethyl ether for 6 h. During this period the mixture was agitated every 15 min intervals and following filtration, diethyl ether was removed using an evaporator (60 °C). 0.3 g of dried extract were added to 99.7 ml of sterile distilled water to obtain 0.3% extract.

Parsley extract: parsley plant was purchased from a local

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market, and washed in distilled water. Amount of 100g was ground for 2 minutes without addition of water then filtered by thieving through cotton cloth under pressure. 0.3 ml of the pure extract were added to 99.7 ml of sterile distilled water to obtain 0.3% parsley extract.

C. Sensory Analysis

Sensory characteristics and overall acceptability of *Lucioperca Lucioperca* fillets were assessed by a panel of six experienced panelists on the basis of a 10-point scale of each sample. Sensory characteristics studied included general appearance, odor and texture of fish. Scale employed for evaluating sensory quality was developed based on the guidelines given by [10]. 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 [11]. A score less than 4 indicate that the fish is rejected.

D. Biochemical Parameters

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 [12]. Determination of total volatile basic nitrogen (TVB-N) content of fish was determined according to the method of [13]. TVB-N contents were expressed as mg nitrogen/ 100 g of fish sample. Thiobarbituric acid (TBA) assay was carried out according to the procedure of [14]. Fish sample (10 g) was mixed with 25 ml of 20% trichloroacetic acid (w/v) and homogenized in a blender for 30 s. After filtration, 2 ml of the filtrate were added to 2 ml of 0.02 M aqueous TBA in a test tube. The test tubes were incubated at room temperature in the dark for 20 h; then the absorbance was measured at 532 nm by using UV–vis spectrophotometer (model UV-1200, Shimadzu, Japan). TBA value was expressed as mg malonaldehyde (MA) per kg of fish sample.

Biogenic amines were extracted according to [15] using butanol: chloroform mixture (1:1v/v), then dansylated using amine-2HCl. The results were detected by HPLC (system, Agilent Technologies, Waldbronn, Germany, model G 1311A).

E. Bacteriological parameters

Psychrophilic bacteria were counted per gram using plate count agar medium. The plates were inoculated and incubated at 4°C for 5-7 days as described [16]. *Enterobacteriaceae* counts (EBC) were enumerated by the pour plating method on Violet Red Bile Glucose Agar (VRBGA; Difco, Detroit, Michigan, USA). The plates were overlaid with a virgin layer of the same growth medium before incubation at 37 °C for 24h [17]. *Pseudomonas* were enumerated on *Pseudomonas* Agar Base (CM 559; Oxoid) supplemented with cetrinide, fucidin, and cephaloridine (CFC) supplements (SR 103; Oxoid, Basingstoke, Hampshire, UK) providing a selective isolation medium for *Pseudomonas* spp. Colonies were counted after 2-days incubation at 25 °C.

F. Statistical Analysis

The differences among means were significant at significance level of $P < 0.05$ using Tukey test as a post-hoc test. All statistics were run on the computer using SAS program (SAS 2000, Version 6.12, SAS Institute Incorporation, Cary, NC).

III. RESULTS AND DISCUSSION

Organoleptic assessment is a good tool for evaluating the freshness of all fish species. It is simple, fast, costless, and provides immediate quality information. The acceptability of fish products during frozen storage depends on the changes in their sensory attributes. Fish samples were considered to be acceptable for human consumption until the sensory score reached 4 [18]. Figure (1) shows a summary of the organoleptic results. All examined groups at zero time were in fresh manner and had high scores ranged from 9.1 to 9.4. This indicates that all samples at start of experiment were in excellent quality. On the 3rd day of chilling there was no significant changes on organoleptic parameters. By the 6th day, there was a significant loss in the fish quality for all samples, which were rejected by the panelists after 6, 12, 15 and 12 days for control, green tea, coriander sativum and parsley treated groups, respectively. Our results coincide with [19]. they found that flavor and texture of the whole crucian carp treated with tea polyphenols (0.2% TP) were improved during storage at $4 \pm 1^\circ\text{C}$.

The initial pH values were 6.51, 6.50, 6.48 and 6.51 for control, green tea, coriander sativum and parsley treated groups, respectively Fig. (2). There was no significant effect between control and treated groups at zero time. Meanwhile, on 3rd and 6th days of storage the significant effect of different treatments was noticed.

The pH values of control samples were increased reaching 6.8 after 6 days of storage. This increase in the pH is attributed to the enzymatic degradation of the fish muscles and the production of volatile basic alkaline components (e.g., ammonia and trimethylamine) by spoilage bacteria [20].

Gradual increases in pH were noticed at 9th day where pH became 6.84 ± 0.12 , 6.72 ± 0.11 and 6.85 ± 0.15 in green tea, coriander sativum and parsley treated groups respectively. This may be due to the phenolic compound on natural extracts which can enhance microbial inhibition and contributes to the extending of the preservation of fish samples for inhibiting the activity of the endogenous proteases.

The Primary value of TVB-N in control and treated groups ranged from 10.1 to 11 mg/100g. There was no significant difference in 0 day between them ($p > 0.05$). On the 6th day TVB-N value in control, green tea, coriander sativum and parsley treated groups was 19.5, 17.4, 12.14 and 13.75 (mg TVB-N/100 g), respectively. The significant effect of natural extracts appeared ($p < 0.05$) on 6th day. TVB-N is mostly created due to bacteria decomposition of fish flesh; increase of bacteria load during the period is considered a reason for deterioration [21]. All treated groups in addition to control did not exceed maximum acceptable value of TVB-N reported 25 mg of nitrogen for 100 g of fish meat [21].

In zero days, the mean value of TBA was 0.39 ± 0.08 , 0.35 ± 0.09 , 0.31 ± 0.07 and 0.34 ± 0.09 mg MAD/kg in control, green tea, coriander sativum and parsley treated groups, respectively. There were no meaningful differences between all groups. Low value of TBA represents the good quality of fish. Usually, the value of 1-2 mg MAD/kg is considered as acceptable limit for TBA so that upper than this value; undesirable smell will be created in fish [22]. Gradual increments were observed in control, green tea, coriander sativum and parsley treated groups to reach 2.23 ± 0.11 , 1.37 ± 0.10 , 1.11 ± 0.09 and 1.29 ± 0.13 mg MAD/kg, respectively. All treated groups were significant on control group ($P < 0.05$) by the end of storage time. Thiobarbituric acid was in acceptable limit, in all treated groups meanwhile, control group was upper than acceptable limit. The coriander sativum extract act as a best natural protector against oxidative rancidity; these results coincide with [23] who reported that the methanolic extracts of coriander fruits showed better antioxidant activity. Antioxidant activities of extracts play an important role to neutralize free radicals and disable the single and triple oxygen [24].

TABLE (1) EFFECT OF NATURAL EXTRACTS ON AEROBIC PLATE COUNTS (APC) $\text{LOG}_{10}\text{CFU/G}$ DURING CHILLING DAYS AT $4 \pm 1^\circ\text{C}$

	Control	Green tea	Coriander sativum	Parsley
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Zero day	4.5 ± 0.32^a	4.12 ± 0.34^a	3.98 ± 0.22^b	4.19 ± 0.29^a
3 days	5.1 ± 0.24^a	4.59 ± 0.29^b	4.12 ± 0.37^c	4.6 ± 0.31^b
6 days	6.4 ± 0.41^a	5.12 ± 0.43^b	4.95 ± 0.28^b	5.2 ± 0.34^b
9 days	7.8 ± 0.51^a	6.08 ± 0.47^{bc}	5.8 ± 0.36^c	6.14 ± 0.38^b
12 days	8.7 ± 0.64^a	7.1 ± 0.54^{bc}	6.51 ± 0.49^c	7.21 ± 0.48^b
15 days	9.87 ± 0.76^a	8.09 ± 0.65^b	7.2 ± 0.58^c	8.14 ± 0.64^b

Mean values with different letters in the same row represent significant differences ($p < 0.05$).

TABLE (2) EFFECT OF NATURAL EXTRACTS ON PSEUDOMONAS COUNT $\text{LOG}_{10}\text{CFU/G}$ DURING CHILLING DAYS AT $4 \pm 1^\circ\text{C}$

	Control	Green tea	Coriander sativum	Parsley
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Zero day	3.1 ± 0.17^a	2.8 ± 0.19^a	2.82 ± 0.21^a	2.9 ± 0.18^a
3 days	4.3 ± 0.21^a	3.5 ± 0.24^b	3.4 ± 0.28^b	3.54 ± 0.27^b
6 days	5.1 ± 0.37^a	4.2 ± 0.36^b	4.25 ± 0.34^b	4.28 ± 0.41^b
9 days	5.9 ± 0.51^a	4.8 ± 0.51^b	4.76 ± 0.41^b	4.78 ± 0.45^b
12 days	6.4 ± 0.41^a	5.5 ± 0.49^b	5.78 ± 0.48^b	5.4 ± 0.53^b
15 days	7.17 ± 0.53^a	6.13 ± 0.47^b	6.3 ± 0.53^b	5.98 ± 0.42^b

Mean values with different letters in the same row represent significant differences ($p < 0.05$).

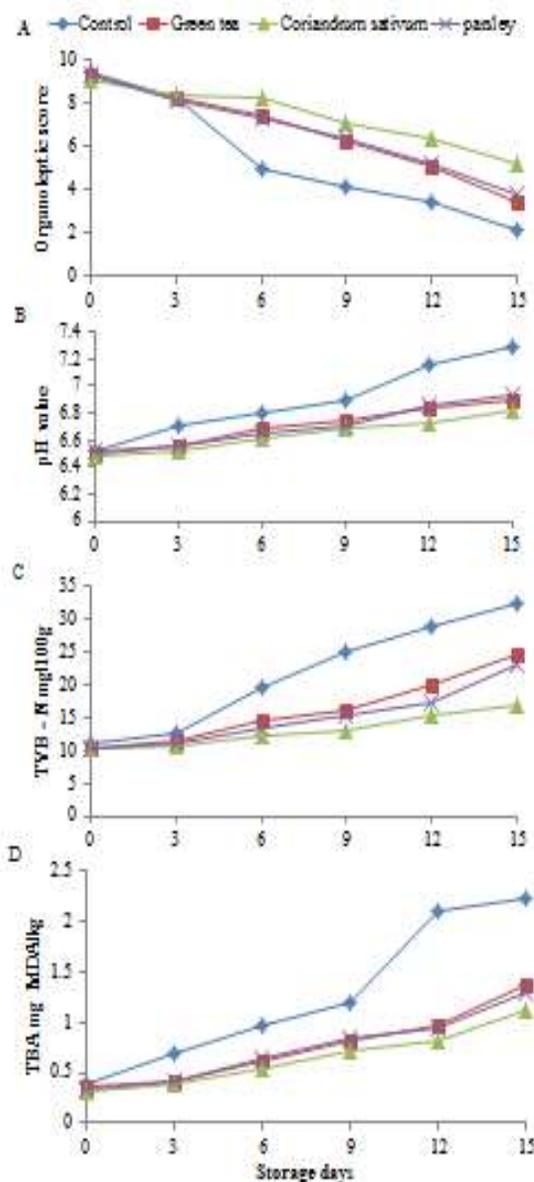


Fig. (1) effect of natural extracts on (A) organoleptic score, (B) pH value (C) TVB-N, (D) TBA during storage periods at $4 \pm 1^\circ\text{C}$

The Primary value of APC in the present paper for all groups was ranged from 3.98 ± 0.22 to 4.5 ± 0.32 log_{10} cfu/g that represented high quality of studied fillet. The microbial load of freshwater fishes were changed related to various factors such as water situation and temperature of farming environment, authors proposed the primary APC value (2-6 log CFU/g) for various species of fresh water fish [25],[26]. At zero time the significant effect was only noticed in coriander sativum treated group ($p < 0.05$) table (1). Progressive increases in all groups and significant effects appeared on all treated groups ($p < 0.05$) on comparing with control at all storage days. The Maximum acceptable values of total viable count of APC for fishes are 7 log_{10} cfu/g that was proposed by international commission on microbiological specifications for foods [27]. The control group was accepted microbiologically until the 6th day of

storage. Green tea and parsley treated groups until 12th days, meanwhile CS until 15th day were accepted microbiologically.

TABLE (3) EFFECT OF NATURAL EXTRACTS ON ENTEROBACTERIACEAE COUNTS LOG₁₀CFU/G DURING CHILLING DAYS AT 4±1OC

	Control	Green tea	Coriander sativum	Parsley
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Zero day	2.4 ± 0.12 ^a	2.1 ± 0.11 ^a	2.12 ± 0.14 ^a	2.16 ± 0.13 ^a
3 days	2.8 ± 0.15 ^a	2.4 ± 0.13 ^b	2.38 ± 0.17 ^b	2.43 ± 0.14 ^b
6 days	3.1 ± 0.21 ^a	2.42 ± 0.19 ^b	2.46 ± 0.27 ^b	2.61 ± 0.25 ^b
9 days	3.5 ± 0.31 ^a	2.7 ± 0.25 ^{bc}	2.65 ± 0.29 ^b	2.74 ± 0.31 ^b
12 days	3.8 ± 0.28 ^a	2.9 ± 0.31 ^{bc}	2.95 ± 0.28 ^b	2.88 ± 0.34 ^b
15 days	4.2 ± 0.29 ^a	3.14 ± 0.35 ^b	3.2 ± 0.37 ^b	3.21 ± 0.37 ^b

Mean values with different letters in the same row represent significant differences (p < 0.05).

TABLE (4) EFFECT OF NATURAL EXTRACTS ON BIOGENIC AMINES (MG/ KG) DURING CHILLING DAYS AT 4±1OC:

		Control	Green tea	Coriander sativa	Parsley
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Zero day	Histamine			1.45 ± 0.5	
	Cadaverine			2.73 ± 0.67	
	Putrescine			0.28 ± 0.1	
6 th day	Histamine	3.45±0.78	1.73±0.48	2.24±0.5	2.48±0.62
	Cadaverine	120.95±13.94	83.45±12.21	70.15±11.6	93.13±12.53
	Putrescine	62.1±9.54	42.2±7.93	33.5±5.4	48.43±8.43
15 th day	Histamine	22.4±3.79	10.75±2.37	14.11±2.85	15.6±3.01
	Cadaverine	306.5±86.44	196.16±42.1	153.25±25.47	214.55±49.45
	Putrescine	180.7±40.9	115.3±14.1	86.7±12.87	133.7±14.77

Mean values with different letters in the same row represent significant differences (p < 0.05).

As shown in table (3) the concentration of Enterobacteriaceae was initially very low, starting around 2-2.4 log₁₀cfu/g in all sample groups with the final count at the end of the storage 4.2± 0.29 in the control group which is relatively high. However, the contribution of Enterobacteriaceae to the microbiota of fish and its spoilage potential must be taken into consideration especially in the case of polluted water or a delay in chilling after catch [31]. There is a significant decrease (P < 0.05) in Enterobacteriaceae count of all treated groups than those of control group, although, the lowest Enterobacteriaceae concentration detected in the samples treated with Green Tea with a mean 3.14 ± 0.35, there is no significant difference among Enterobacteriaceae counts of all treated groups.

During the storage period (15 days), biogenic amines; Histamine, cadaverine and putrescine had been estimated in fish fillets stored at 4°C. As shown in table (4) Histamine remained at low levels during the storage, then slightly increased till reached 22.4±3.79 mg/ kg at the end of the period, which is still under the permissible limits (50mg/ kg) according to the FDA [32], but slightly above the Egyptian legal permissible limits (20mg/kg) [33]. However, significant increases (p < 0.05) of Putrescine and Cadaverine were observed in all the control samples during storage. For example, concentrations of Putrescine and Cadaverine greatly increased 180.7±40.9 and 306.5±86.44 at the end of storage

The pseudomonas count ranged from 2.8 ± 0.19 to 3.1 ± 0.17 log₁₀cfu/g. table (2) there was no significant effect related to addition of natural extracts at zero time. The microbial population of fish stored aerobically under chilling condition consists almost exclusively of Pseudomonas spp. [28]. Pseudomonas spp. count progressively increased with further storage days. A significant difference between control and treated groups (p < 0.05) obtained from 3rd day of storage to the end of storage period. Nearly the same counts of pseudomonas spp. during fish chilling were detected by [29]. By the end of storage period the highest reduction count achieved belongs to parsley treated group (1.19 log₁₀cfu/g). This may be due to Phenolic components extracted from parsley are liable for their antimicrobial and antioxidant activities. Apiol, Meristici, Alpha Pinene and beta Pinene are from these components [30].

period from 0.28 ± 0.1 and 2.73 ± 0.67 mg. kg⁻¹ at the initial time, respectively. The groups of treated samples showed decreases in the contents of all biogenic amines tested throughout storage in comparison with the control ones, as shown in table (4), samples treated with coriander sativum extract prevailed lowest putrescine and cadaverine levels while the lowest histamine levels appeared in samples treated with green tea due to its content of flavonoids which is natural antioxidant as well as possess of histamine-reducing effect.

The analysis of data regarding the present paper shows that adding natural extracts on *Lucioperca Lucioperca* fillets causes of delay microbial and oxidative deterioration and it increases the shelf life by 6 days over the control samples in case of GT and parsley extracts while, CS extend storage time by 9 days over the control.

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