Animal Health Research Journal Vol. 7, No. 5, December 2019 pp. 68-80

ISSN: 2356-7767

Assessment the impact of green tea and rosemary nanoemulsion on the shelf life of fish fillets.

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Received in 6/11/2019
Accepted in 11/12/2019

Abstract
The present study was designed to investigate antioxidant and antimicrobial activity of green tea and rosemary nanoemulsion on Nile tilapia (*Orechromis niloticus*) fillets samples during refrigerated storage at 4 ± 1°C. Fish fillets samples were divided into 5 groups, the 1st one was untreated (control), the 2nd and 3rd group were dipped into green tea nanoemulsion (0.5% - 1%) for 30 minutes and the 4th and 5th groups were dipped in rosemary nanoemulsion (0.5% - 1%) for 30 minutes, respectively. The fish samples were analyzed periodically for sensory evaluation, microbiological [Aerobic plate count (APC) and total psychrotrophic count] and chemical [pH, Total Volatile Basic Nitrogen (TVB-N), Tri-methyl Amine (TMA) and Thiobarbituric acid (TBA)]. The results indicated that dipping treatments retard the spoilage of fish compared to untreated fish fillets. Dipping treatment in 1% green tea nanoemulsion was more efficiently inhibited the increase APC and total psychrotrophic count followed by rosemary 1%, green tea 0.5% and rosemary 0.5%, respectively. Dipping treatments predominantly reduced chemical spoilage, reflected in TVB-N, pH, TBA and TMA and increased the overall sensory quality of fish fillets compared to untreated fillets. The sensory, microbiology, and chemical results indicated that green tea nanoemulsion has strong antimicrobial and antioxidant activity and can maintain the quality parameters and extend the shelf life of refrigerated Nile tilapia fillets samples for 5 days longer than control one, while the rosemary nanoemulsion extend the shelf life for 4 days longer than control one.

Keywords: *Green tea rosemary, nano emulsion, fish fillets, antimicrobial, sensory, TBA, TVB, N, TMA, shelf life.*

Introduction

Fish have rich source of essential nutrients required for supplementing both infant and adult diets. Due to high content of polyunsaturated fatty acid and subsequently fish meat is highly prone to oxidative rancidity resulting spoilage, the oxidation of polyunsaturated fatty acids is catalyzed by heat, light, and enzymes, and leads to the formation of peroxides, aldehydes, ketones, and free radicals, also, fish flesh and fish oil is beneficial in reducing the serum cholesterol. The protein content of fish is also important when considering the quality. Fish meat is also a rich source of minerals and the most abundant micro-elements are Zinc (Zn), Iron (Fe) and Copper (Cu). Spoilage of fish resulted from changes brought through biological reactions such as oxidation of lipids, the activities of the fish muscular enzymes and the metabolic activities of microorganisms. Chemical and enzymatic reactions are usually responsible for the initial loss of freshness whereas, microbial activity is responsible for the spoilage and thereby establishes product shelf life **(Arashisara *et al.,* 2004).**

Natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones; thus they are more readily acceptable to the modern consumers **(Almey *et al.,* 2010).**

Green tea has antioxidative effects 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, the major antibacterial polyphenol compounds are the catechin groups**.** Green tea is known to have the highest antioxidant capacity compared to other tea types **(Vinson, 2000)** and has greater antioxidant effect than vitamin C, vitamin E, BHA (Butylated hydroxyanisole) and BHT (Butylated hydroxytoluene). Green tea is one of the natural preservatives applied to many foods as antioxidant and antimicrobial agents **(Kristanti and Punbusayakul, 2009).**

Extracts of green tea are rich in flavanols and their gallic acid derivatives, namely, catechin, epicatechin, gallocatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate (**Rice-Evans *et al.,* 1995** and **Johnson *et al.,* 2010).** In addition, they contain a range of natural flavor-rich components such as terpenes, oxygenated terpenes, sesquiterpenes and organic acids. Green tea is one of the natural preservatives applied to many foods as antioxidant and antimicrobial agents **(Kristanti and Punbusayakul, 2009).** Also, **Li *et al.* (2012) and Hussein *et al.* (2016)** proved that green tea and rosemary were improved flavor and texture of the treated carp during storage at 4±1°C.

Rosemary has been reported to contain certain compounds such as rosmanol, rosmaridiphenol, rosmariquinone, and carnosol, which may be up to four times equal to butylated hydroxyanisole (BHA) and as effective as butylated hydroxyltoluene (BHT) as antioxidants **(Cadun *et al.,* 2008** and **Rohlík *et al.,* 2013).** Rosemary has antimicrobial activity against both Gram-positive and Gram-negative bacteria due to the presence of phenolic compounds, carnosic acid and carnosol **(Offord *et al.,* 1997** and **Del Campo *et al.,* 2000).**

Manufacture of nanomaterial involves the approaches like 'top down' or 'bottom up'. Where top down manufacturing of nanomaterials involves breaking down larger particles of matter to particles of only nanometers in dimension by physical or chemical means. Mechanical milling or grinding is an important method to convert the macro sized particles to the nanoscale. This technique is commonly used in the enhancement of binding ability of product ingredients like wheat flour. Some researchers have also indicated it as a method to enhance the antioxidant properties of ingredients by increasing the surface area, where reduction in size increases the active sites as well as the surface area of active principles present in the materials. As the powder size of green tea is reduced to 1000 nm by dry milling, the high ratio of nutrient digestion and absorption resulted in an increase in the activity of an oxygen-eliminating enzyme **(Shibata, 2002).**

Nanoemulsion, with droplet sizes ranging from 10 to 200 nm, are one of the successful systems that can be used as carriers or delivery systems for lipophilic compounds, such as flavors, drugs, antioxidants, and antimicrobial agents **(Rao and McClements, 2011** and **Weiss *et al.,* 2008).** Nanoemulsion consists of an oil phase dispersed in an aqueous continuous phase, with each oil droplet surrounded by a thin interfacial layer of surfactant molecules, which help in stabilizing the nanoemulsion system to a more stable formulation **(Borrin *et al.,* 2016).** Transformation of essential oils into nanoemulsion increases their antimicrobial effect (**Otoni *et al.,* 2014**).

Edible coatings from nanoemulsion would represent an effective approach to place active ingredients such as essential oils on the surface of foods. Embedding essential oils nanoparticles within edible coatings could be an interesting strategy to extend the shelf-life of food **(Mohammad *et al.,* 2017).**

The aim of this study was to take the advantages of green tea and rosemary properties as antioxidant and antimicrobial agents to prepare and characterize nanoemulsion and study the effect of green tea and rosemary nanoemulsions coatings on quality and shelf life of fish fillets.

Materials and Methods

1- Preparation of green tea and rosemary nanoemulison

1.1- Extraction of essential oils: It was carried out according to the technique recommended by **AOAC (2000).**

1.2- Preparation of nanoemulison: It was carried out according to the technique recommended by **(Forgiarini *et al.,* 2001)**.

1.3- Transmission electron microscopy (TEM):

The morphology and size of nanoemulsions were analyzed using TEM with negative staining of phosphor tungstic acid (PTA 1%) according to **(Kaur *et al.,* 2016).**

2- Fish samples preparation:

About 15 kilograms Nile tilapia (Orechromis niloticus) fish samples were purchased from a fish market in Cairo, Egypt at the same day of harvesting. Each sample was gutted, deheaded, cleaned and filleted into two pieces of about 100 g weight for each piece. Then the samples were wrapped in sterile polyethylene bags and directly transferred to the laboratory in sterile icebox for further preparation within 2 h after purchasing. The samples were divided into 5 groups; the 1st group was untreated control (C), while the 2nd group was dipped into green tea nanoemulion 1% for 30minutes, the 3rd group dipped into green tea nanoemulion 0.5% for 30 minutes, the 4thgroup dipped into rosemary nanoemulion 1% for 30 minutes and 5th group dipped into rosemary nanoemulion 0.5% for 30 minutes, then the dipping solutions were discarded. Treated fish fillets samples were labeled and packaged as triplicates inside fiber dishes, stored at 4 ± 1^{o}C inside the refrigerator and were subjected to sensory, physicochemical and microbiological assessment at day zero (within 2 hours after treatment) then periodically every three days until decomposition.

3- Sensory evaluation:

Sensory evaluation of Nile tilapia fillets were assessed by a panel of six experienced panelists on the basis of a 10-point scale of each sample. Sensory evaluation was based on the various sensory characters as odor, texture and general appearance of fish (**Hussein *et al.,* 2016)**. 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. A score less than 4 indicate that the fish is rejected.

4- Microbiological examination:-

The microbiological quality of treated and control fillet samples was assessed on the basis of aerobic plate count (APC) and total psychrotrophic count as per the procedure of **APHA (2001)**.

5- Chemical analyses:-

5.1- Determination of pH: The technique was carried out according to the technique recommended by **AOAC (1990).**

5.2- Determination of Total Volatile Basic Nitrogen (TVB-N): The technique was carried out according to the technique recommended by **ES: 63-9 (2006**),

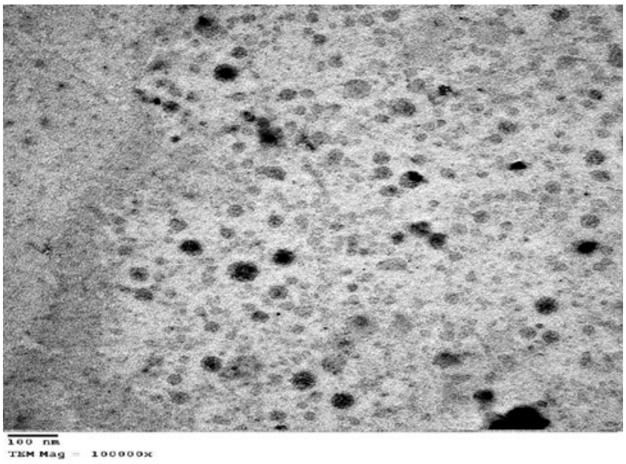
5.3- Determination of thiobarbituric acid (TBA): The technique was carried out according to the technique recommended by **ES: 63-10 (2006**).

5.4- Trimethylamine (TMA): The technique was carried out according to the technique recommended by **AOAC (1978)** .

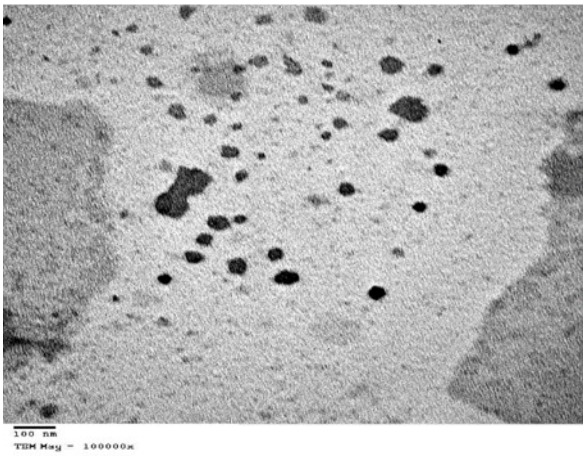
6- Statistical analysis.

Statistical analyses were run in triplicate and results were reported as mean values ± standard deviation (SD). Data were subjected to analysis of variance (one-way ANOVA Excel 5.0). A p-value less than 0.05 ($p \leq 0.05$) was considered statistically significant.

Results and discussion



(a)



(b)

Fig. (1): Transmission electron microscope micrographs of (a) green tea and (b) rosemary nano emulsion

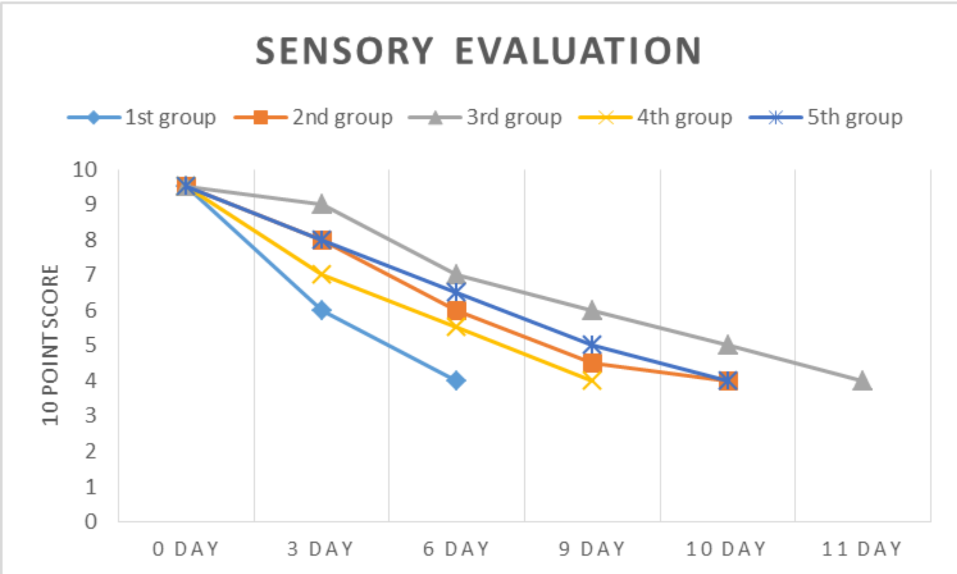


Fig. (2): The effects of various concentrations of green tea and rosemary nanoemulsion on overall acceptability of the examined fish fillet samples stored at 4±1ºC.
(the final score is 10–9 for excellent, 8–7 for good, 6–5 for fair and acceptable, 4–3 for poor and 2–1 for very poor A score less than 4 indicate that the fish fillets samples is rejected)

Table (1). Mean values of APC of treated and non-treated samples (mean $\log_{10}$ cfu/g ± SD of 3 trials) during storage at 4±1°C.

	1st group (control)	2nd group (0.5%GT)	3rd group (1%GT)	4th group (0.5%RM)	5th group (1%RM)
0 day	3.46^{a}±0.07	3.26^{a} ±0.06	3.08^{b}±0.04	3.38^{a} ±0.05	3.23^{b} ±0.03
3 day	4.92^{a} ±0.09	4.20^{b} ±0.05	3.71^{c}±0.06	4.43^{d}±0.07	4.04^{b} ±0.05
6 day	6.00^{a} ±0.09	5.00^{b} ±0.12	4.45^{c} ±0.08	5.38^{d} ±0.09	4.97^{b} ±0.08
9 day	S	5.76^{a} ±0.06	5.15^{b} ±0.12	5.88^{a} ±0.05	5.69^{a}±0.06
10 day	S	5.90^{a} ±0.07	5.60^{b} ±0.08	S	5.95^{a} ±0.09
11 day	S	S	6.00 ±0.14	S	S

Values are expressed as mean ± SD with different alphabetical superscript along row are significantly different at ($p < 0.05$). GT= green tea RM= rosemary S= spoiled.

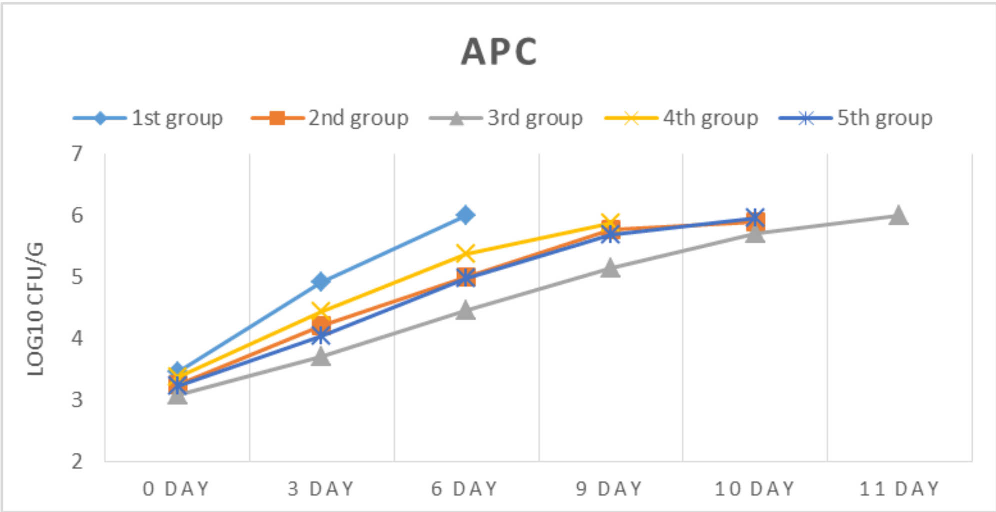


Fig. (3): Changes in aerobic plate count (APC) of treated and non-treated fish fillet stored at 4±1°C

Table (2). Mean values of total psychrotrophic count of treated and non-treated samples (mean $\log_{10}$ cfu/g ± SD of 3 trials) during storage at 4±1°C:

	1st group (control)	2nd group (0.5%GT)	3rd group (1%GT)	4th group (0.5%RM)	5th group (1%RM)
0 day	$2.80^{a}\pm0.03$	$2.71^{a}\pm0.03$	$2.68^{a}\pm0.04$	$2.78^{a}\pm0.03$	$2.7^{a}\pm0.04$
3 day	$3.76^{a}\pm0.05$	$3.15^{b}\pm0.03$	$3.00^{b}\pm0.03$	$3.68^{a}\pm0.06$	$3.15^{b}\pm0.04$
6 day	$4.86^{a}\pm0.05$	$3.98^{b}\pm0.06$	$3.73^{c}\pm0.05$	$4.08^{b}\pm0.08$	$3.88^{b}\pm0.05$
9 day	S	$4.69^{a}\pm0.05$	$4.36^{b}\pm0.12$	$4.87^{a}\pm0.09$	$4.62^{a}\pm0.07$
10 day	S	$5.15^{a}\pm0.09$	$4.83^{b}\pm0.07$	S	$5.04^{a}\pm0.09$
11 day	S	S	5.14 ± 0.15	S	S

Values are expressed as mean ± SD with different alphabetical superscript along row are significantly different at ($p < 0.05$). GT= green tea RM= rosemary S= spoiled.

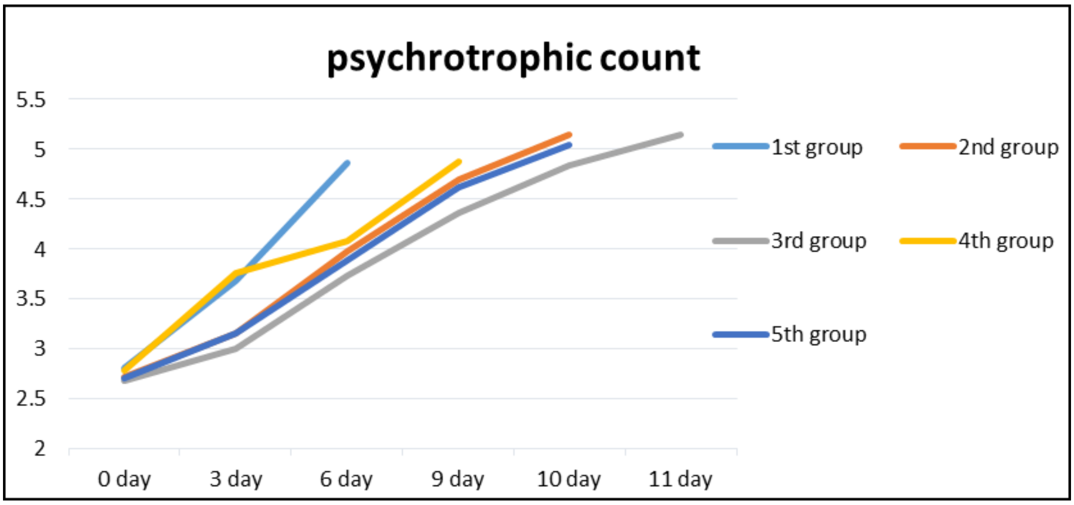


Fig. (4): Changes in Total Psychrotrophic count of treated and non-treated fish fillet stored at 4±1°C.

Table (3). Mean values of pH of treated and non-treated samples (mean log1010 cfu/g ± SD of 3 trials) during storage at 4±1°C:

	1st group (control)	2nd group (0.5%GT)	3rd group (1%GT)	4th group (0.5%RM)	5th group (1%RM)
0 day	6.28^{a}±0.04	6.24^{a} ±0.02	6.18^{a} ±0.04	6.22^{a} ±0.03	6.16^{a} ±0.02
3 day	6.42^{a} ±0.08	6.33^{a} ±0.05	6.23^{a} ±0.07	6.34^{a} ±0.04	6.24^{a} ±0.09
6 day	6.59^{a} ±0.05	6.38^{b}±0.05	6.27^{b} ±0.04	6.48^{b} ±0.09	6.36^{b} ±0.15
9 day	S	6.42^{a} ±0.09	6.32^{a} ±0.09	6.62^{b} ±0.07	6.41^{a} ±0.12
10 day	S	6.56^{a} ±0.11	6.44^{a} ±0.07	S	6.56^{a} ±0.08
11 day	S	S	6.52 ±0.09	S	S

Values are expressed as mean ± SD with different alphabetical superscript along row are significantly different at ($p < 0.05$). GT= green tea RM= rosemary S= spoiled

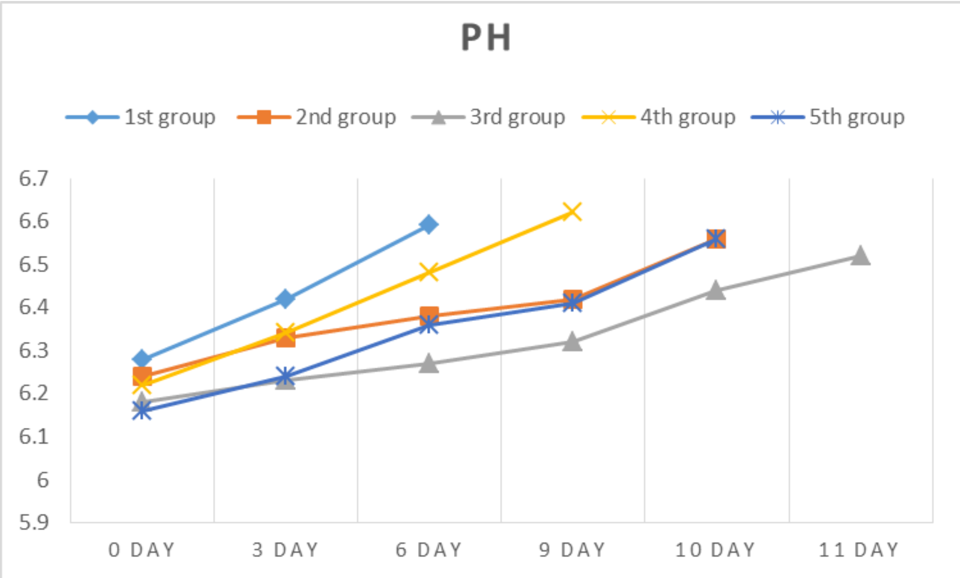


Fig. (5): Changes in PH of treated and non-treated fish fillet stored at 4±1°C

Table (4). Mean values of TVB-N of treated and non-treated samples (mean of 3 trials ± SD) during storage at 4±1°C:

	1st group (control)	2nd group (0.5%GT)	3rd group (1%GT)	4th group (0.5%RM)	5th group (1%RM)
0 day	18.2^{a} ±0.09	18.2^{a} ±0.09	18.2^{a} ±0.09	18.2^{a} ±0.09	18.2^{a} ±0.09
3 day	23.8^{a} ±0.12	22.9^{b}±0.08	21.5^{c}±0.14	23.8^{d}±0.07	22.4^{f}±0.11
6 day	32.2^{a} ±0.15	25.2^{b} ±0.12	24.3^{c} ±0.09	26.6^{d} ±0.11	25.2^{b} ±0.08
9 day	S	26.6^{a} ±0.09	26.1^{b} ±0.13	29.4^{c} ±0.09	27^{d} ±0.21
10 day	S	30.3^{a} ±0.15	28^{b} ±0.09	S	28.5^{c} ±0.13
11 day	S	S	29.8 ±0.14	S	S

Values are expressed as mean ± SD with different alphabetical superscript along row are significantly different at ($p < 0.05$). GT= green tea RM= rosemary S= spoiled.

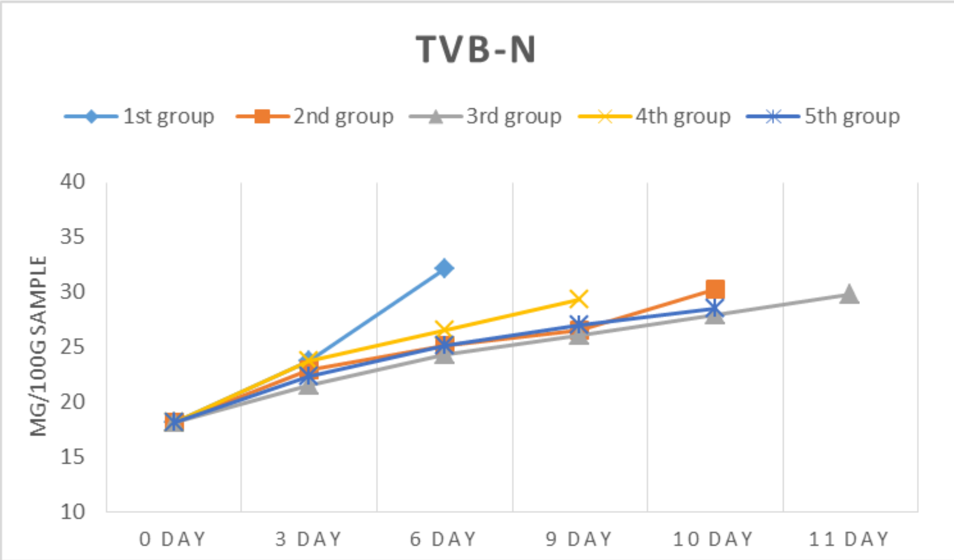


Fig. (6): Changes in TVB-N of treated and non-treated fish fillet stored at 4±1°C.

Table (5). Mean values of TMA of treated and non-treated samples (mean of 3 trials ± SD) during storage at 4±1°C:

	1st group (control)	2nd group (0.5%GT)	3rd group (1%GT)	4th group (0.5%RM)	5th group (1%RM)
0 day	0.9^{a}±0.06	0.9^{a} ±0.06	0.9^{a} ±0.06	0.9^{a} ±0.06	0.9^{a} ±0.06
3 day	3.8^{a} ±0.09	1.6^{b}±0.05	1.3^{c}±0.06	1.8^{b} ±0.05	1.5^{b} ±0.07
6 day	6.9^{a} ±0.09	4.3^{b} ±0.15	3.4^{c}±0.09	4.1^{b} ±0.09	3.8^{f} ±0.09
9 day	S	6.6^{a} ±0.12	5.3^{b} ±0.07	6.9^{c}±0.08	5.7^{d} ±0.09
10 day	S	7.3^{a} ±0.08	6.1^{b} ±0.09	S	6.4^{c} ±0.15
11 day	S	S	7.2 ±0.12	S	S

Values are expressed as mean ± SD with different alphabetical superscript along row are significantly different at ($p < 0.05$). GT= green tea

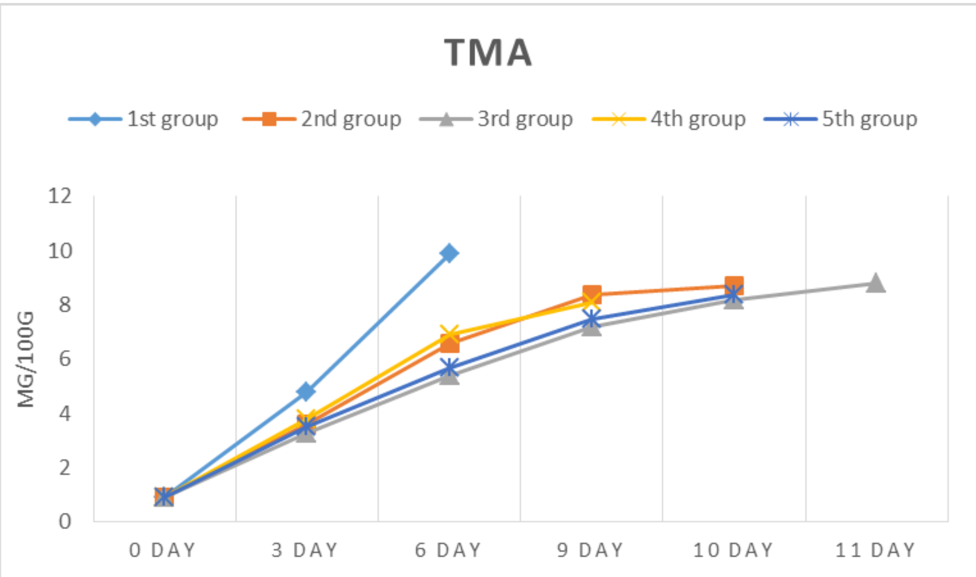


Fig. (7): Changes in (TMA) of treated and non-treated fish fillet stored at 4±1°C

Table (6). Mean values of TBA of treated and non-treated samples (mean of 3 trials ± SD) during storage at 4±1°C:

	1st group (co (Control)	2nd group (0.5%GT)	3rd group (1%GT)	4th group (0.5%RM)	5th group (1%RM)
0 day	0.48ᵃ±0.02	0.48 ᵃ ±0.02	0.48 ᵃ ±0.02	0.48 ᵃ ±0.02	0.48 ᵃ ±0.02
3 day	0.71 ᵃ ±0.02	0.66 ᵃ ±0.03	0.54 ᵃ ±0.01	0.69 ᵃ ±0.01	0.61 ᵃ ±0.01
6 day	1.06 ᵃ ±0.03	0.85ᵇ±0.03	0.60ᶜ±0.02	0.81 ᵇ ±0.02	0.73 ᵇ ±0.02
9 day	S	0.97 ᵃ ±0.04	0.71 ᵇ ±0.02	0.96 ᵃ ±0.03	0.89 ᵃ ±0.02
10 day	S	1.05 ᵃ ±0.02	0.82 ᵇ ±0.03	S	0.98 ᵃ ±0.03
11 day	S	S	0.98 ±0.02	S	S

Values are expressed as mean ± SD with different alphabetical superscript along row are significantly different at ($p < 0.05$). GT= green tea RM= rosemary S= spoiled

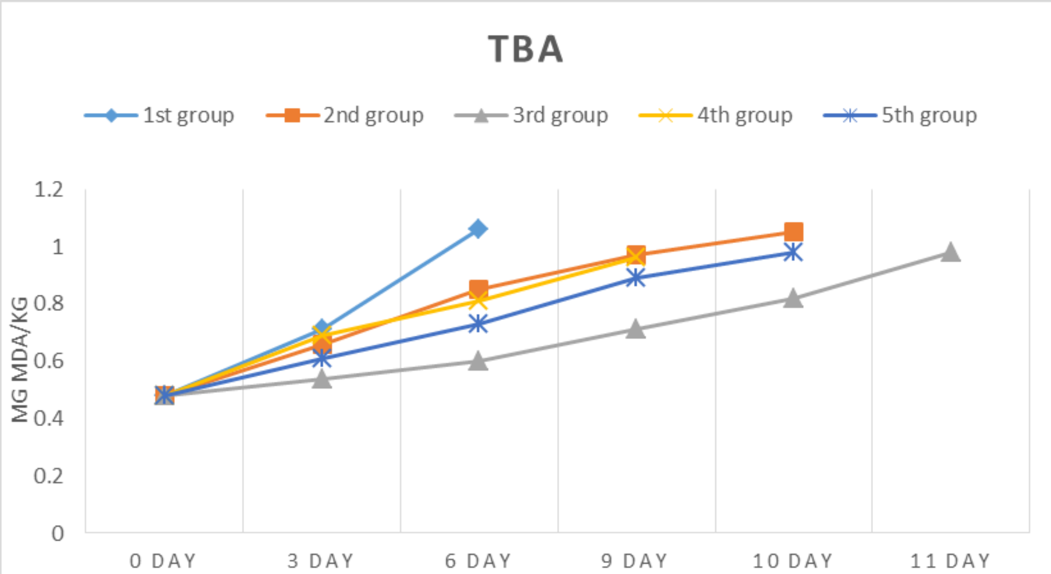


Fig. (8): Changes in (TBA) of treated and non-treated fish fillet stored at 4±1°C.

Morphology of the nanoemulsions was observed using transmission electron microscopy, and the size of the droplets was qualitatively measured to correlate with the particle size analysis. Transmission electron micrographs of green tea nanoemulsion and rosemary nanoemulsion are given in Fig. (1) a and b, images showed that nanoemulsions droplets were spherical in shape and the droplets size were in nanometric range. Moreover, TEM analysis indicated a very close range of droplet diameter, which demonstrates that, the nanoemulsions are highly homogeneous and uniformly distributed.

Sensory evaluation is the most popular way of assessing the freshness of fish. It is fast, simple, and provides immediate quality information. The results of the sensory evaluation of treated and untreated fish fillets samples during refrigerated storage at 4±1°C., which were obtained in overall acceptability, are illustrated in Fig.(2). From the results, it could be noticed that at zero time storage, there were no significant differences at ($p < 0.05$) between different treatments in sensory scores for overall acceptability. The overall acceptability for all samples decreased gradually with storage time until reached unacceptable after 6th, 10th, 11th, 9th and 10th day of storage, for 1st, 2nd, 3rd, 4th and 5th group, respectively. The results were in accordance with **Lu *et al.* (2009)** who found that the shelf life of untreated northern snakehead fillets was 7 days according to sensory score. Moreover, **Pal *et al.* (2017)** found the shelf life of the fillet treated with tea extracts is adjudged 12 days for refrigerated storage.

Green tea is one of the natural preservatives applied to many foods as antioxidant and antimicrobial agent **(Kristanti and Punbusayakul, 2009).** The aerobic bacterial counts from all tested samples are presented in Table (1) and fig. (3). The initial number of bacteria in Nile tilapia fish fillet samples was 3.46±0.07 log_{10} cfu/g which indicated good quality of fish used in this study. Generally, the difference in initial microbial load of fresh water fishes may be due to water condition and temperature. According to available literature reports, the bacterial counts of different fresh water fish species were between 2.00 and 6.00 log_{10} cfu/g **(Chytiri *et al.,* 2004).**

Microbial activity is responsible for spoilage of most sea foods. For this reason, the aerobic plate counts (APC) have been used in mandatory seafood standards in some European Countries, Japan and USA **(Lund, *et al.*, 2000).** Dipping fish fillet samples in green tea (1%) and rosemary (1%) nanoemulsion led to reduction in the initial aerobic bacterial count at zero time of cold storage 4±1°C as compared to control. On zero day The mean APC for control was 3.46±0.07 log_{10} cfu/g, while after treatment with green tea nanoemulsion 0.5% & 1%, and rosemary nanoemulsion 0.5% & 1%, the mean APC recorded 3.26± 0.06, 3.08± 0.04, 3.38± 0.05 and 3.23± 0.0.03 log_{10} cfu/g, respectively. There was no significant difference between control and treated samples with green tea 0.5% and rosemary 0.5% from the beginning of storage period but there was significant difference between control and treated samples with green tea 1% and rosemary 1%. The Maximum acceptable values of APC for fishes are 6 log_{10} cfu/g that was proposed by **(ES:3494/2005).** The control group accepted microbiologically until the 6th day of storage, while the 2nd, 3rd, 4th and 5th treated groups at 10th, 11th, 9th and 10th day, respectively. APC increased with the storage period for all groups with a different rate. As expected, APC values of untreated samples increased at a faster rate than those of all treated ones, indicating antimicrobial effect of green tea and rosemary nanoemulsion, which confirms in previous studies recorded by **Kim *et al.* (2006) and Chan *et al.* (2007).**

Table (2) and Fig. (4) revealed no significant difference at ($p < 0.05$) between control sample and treated samples on the initial zero day regarding psychrotrophic count (log_{10} cfu/g ± SD) of the examined samples during refrigerated storage. While the five groups recorded 4.86 ±0.05, 5.15±0.09, 5.14±0.15, 4.87±0.09 and 5.04±0.09 on the 6th, 10th, 11th 9th and 10th day of cold storage for 1st, 2nd, 3rd, 4th and 5th groups, respectively . From the present data it could be concluded that the green tea nanoemulsion was more effective than rosemary nanoemulsion. The antibacterial activity of green tea and rosemary are attributed to presence of phenolic compounds **(Del campo *et al.,* 2000 and Sakanaka *et al.,* 2000).** Nearly similar results were obtained by **Gai *et al.* (2014).**

Table (3) and Fig. (5): In the present study, a gradual increase in pH values with storage time from 6.28 ± 0.04, 6.24 ± 0.02, 6.18 ± 0.04, 6.22±0.03 and 6.16±0.02 at zero day while the 1st control group samples were found spoiled after the 6th day of storage. On the other hand, the 2nd, 3rd, 4th and the 5th treated groups were recorded a pH of 6.56±0.11 at the 10th day, 6.52±0.09 at the 11th day, 6.62±0.07 at the 9th day and 6.56±0.08 at the 10th day of storage, while these groups were found spoiled after 10th, 11th, 9th and 10th day of refrigerated storage at 4±1°C, respectively. Higher pH values of the Control group at the 6th day of storage could be attributed to the increase in volatile bases such as ammonia produced by either microbial or muscular enzymes **(Li *et al.,* 2012).** There in significant differences at ($p < 0.05$) between control and treated samples with green tea and rosemary till the spoilage day of control and treated groups. The decrease in pH value of treated groups could be attributed to the antimicrobial effect of green tea and rosemary. The maximum acceptable limit of pH value (6.5) in fish and fish products recommended by **(ES:3494/2005).** Some authors reported a pH limit of acceptability of 6.8 –7.0 **(Erkan *et al.,* 2011).**

TVB-N and TMA have often been documented as biochemical indices to assess freshness and quality; the biochemical indices are related to the activity of spoilage bacteria and endogenous enzymes. **(ES: 3494/2005)** stated that TVB-N critical limits of 25, 30 and 35 mg N/100 g were established for different groups of fish species and have been proposed as an upper acceptability limit for spoilage initiation for fresh fish and reported TMA values of <10 mg/100 g. Values of TVB-N in all groups are presented in Table (4) and Fig.(6). At the beginning of storage, the initial TVB-N value was 18.2±0.09mg /100 g flesh. There was a progressive increase in TVB-N values with storage duration in all groups to reach 32.2 ± 0.15, 30.3 ± 0.15, 29.8±0.14, 29.4±0.09 and 28.5 ± 0.13 mg N/100 g at 6^{th} day,10^{th} day,11^{th} day 9^{th} day and 10^{th} day of refrigerated storage in control, dippng in green tea nanoemulsion 0.05% and 1% and rosemary nanoemulsion 0.05%, 1% samples, respectively. The findings are in agreement with **Nugraha *et al.* (2012) and pal *et al.* (2017)** where fish without treatment had higher TVB–N level compared to that treated with green tea. TMA is produced by the decomposition of trimethylamine oxide caused by bacterial spoilage and enzymatic activity (**Frangos *et al.*, 2010)**. Table (5) and Fig.(7) showed mean values of TMA of treated and non-treated samples during storage at 4±1°C. At day zero TMA of all groups were 0.9 ± 0.06 mg N/100 g, there was no significant difference in 0 day between them (p>0.05). The mean TMA value of the control group samples recorded 6.9±0.09 at the 6^{th} day of storage, while TMA values of treated samples were recorded 7.3±0.08, 7.2±0.12, 6.9±0.08 and 6.4±0.15 for the 2^{nd}, 3^{rd}, 4^{th} and 5th group samples at 10^{th}, 11^{th}, 9^{th} and 10^{th} day of storage period respectively. All treated groups did not exceed the maximum acceptable value of TMA and the green tea nanoemulsion, 1% was the lowest one. The decrease in TMA value of treated groups may be attributed to the antimicrobial effect of green tea and rosemary. TMA is particularly responsible for the unpleasant odor of spoiled fish, and is a common index of seafood quality. However, the changes in sensory attributes often occur before products are hygienically spoiled **(Franzetti *et al.*, 2002).**

TBA value is an index of lipid oxidation measuring malondialdehyde (MDA) content. MDA formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen **(Fernandez *et al.,* 1997).** In the present study, a gradual increase in TBA values with storage time from 0.48 ± 0.02 at day zero in all samples has been observed to reach 1.06±0.03, 1.05±0.02, 0.98±0.02, 0.96±0.03 and 0.98±0.03mg MDA/kg at 6^{th}, 10^{th}, 11^{th}, 9^{th} and 10^{th} day of refrigerated storage for control, green tea 0.5, 1% and rosemary 0.5, 1%, respectively. Table (6) & Fig. (8) there was significant difference at (p < 0.05) between control sample and treated samples on the end day of storage. The decrease in TBA value of treated groups may be attributed to the antioxidant effect of green tea and rosemary. The antioxidant properties of rosemary extract were previously recorded by **Cadun *et al.* (2008).** Green tea is one of the natural preservatives applied to many foods as antioxidant and antimicrobial agents **(Kristanti and Punbusayakul, 2009).** 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. Concerning the permissible limit of TBA value in fish and fish products (4.5 mg MDA/kg) recommended by (**ES: 3494/2005).**

Conclusion and recommendation

From the previously mentioned data, it could be concluded that the dipping of Nile tilapia fillets into green tea nanoemulsion 0.5 &1% and rosemary nanoemulsion 0.5 & 1% for 30 minutes before refrigeration can retain the quality attributes and extend the shelf life for about 4, 5, 3 and 4 days, respectively, more than control samples during refrigerated storage at 4± 1°C. The antimicrobial and antioxidant activities of green tea and rosemary were recorded without any adverse effect on the sensory acceptability of the treated fillets, improved the microbiological quality properties and delayed the appearance of microbial spoilage in chilled fish fillets, which in turn reflected on the reduction of TVB-N, TMA and TBA values and retarded the deterioration of fish fillets. Green tea and rosemary were reported to have an antioxidant activity with clear reduction in the TBA value in treated fillets than control group. Green tea is more effective than rosemary in extension of shelf life of fish fillets, so it is recommended to be used as natural antimicrobial and antioxidant agent for extension of shelf life of fresh fish fillets.

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