6

Egypt. J. Food Sci. **Vol. 46,** pp. 69- 82 (2018)

Influence of Chitosan Based Coating Incorporating Green Tea and Rosemary Extracts on Physicochemical and Microbial Quality of Tilapia fish (*Oreochromis niloticus*) Fillets under Cold Storage

Zeinab S. Farag[1], Ashraf M. Sharaf [1] and Mohamed K. Morsy[2*]

[1]*Food Industries Department, Faculty of Agriculture, Damietta University, Egypt*

[2]*Food Technology Department, Faculty of Agriculture, Benha University, Egypt*

THE incorporation of natural extracts into edible coatings has the potential to improve the quality and safety of fish products. The aim of this work was to evaluate the effectiveness of chitosan coatings including herb extracts against 4 foodborne pathogens; *Staphylococcus aureus, Escherichia coli, Bacillus cereus,* and *Pseudomonas aeruginosa*, as well their impact on physical and biochemical attributes of Tilapia fish (*Oreochromis niloticus*) fillets during storage at 4±1˚C for 15 days. Green tea extract (GTE) and rosemary extract (RE) at concentrations 2 and 3%, respectively were evaluated against foodborne pathogens compared with chitosan coating (control positive; C^+) and control sample (without coating; C). The active packaging was applied on Tilapia fish fillets and the physical parameters (pH and WHC), freshness indictors (TBA and TVB-N), microbial load, and sensory evaluation (color, odor, and texture) were evaluated. The GTE and RE exhibited a high phenolic content and antioxidant capacity. In GTE and/or RE coated samples, the values of TBA, TVB-N, and pH were lower while the WHC was higher than the control sample (up to 15 days).The microbial load of treated samples with coatings was lower than the untreated samples. The sensory evaluation of color, odor, and texture in coated fish fillets were accepted with a high score over 15 days. The results demonstrated that GTE and RE were more active in preventing protein degradation, retarding lipid oxidation, improving the microbial quality, and extend shelf life of Tilapia fish fillets.

Keywords: Tilapia fish, Chitosan, Green tea extract, Rosemary extract, Oxidative stability, Shelf life.

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most important freshwater fish in Egypt (Ko et al., 2016). It is a good source of polyunsaturated fatty acids, protein, minerals, vitamins, and has health benefits. Tilapia fish is produced in aquaculture in a wide-range, it is economic, availabe and has a good taste, as well as low price (Chen et al., 2015). The global Tilapia aquaculture production in 2018 is estimated ~ 6 million tons while in Egypt is about 665300 tons (Tveteras et al., 2018). However, it decomposes quickly and comparatively has a short shelf life because of high nutrients, neutral pH, and high water activity (Wang et al., 2018). The biochemical reactions and microbial metabolism in fish lead to off flavor, texture, and loss of edibility (Liu et al., 2013). One way to overcome the fish spoilage is using antimicrobials (natural and/or synthetic) dips or sprays of the surface of the fillets (Ahmed et al., 2016). However, in these techniques the antimicrobial activity is limited because of the uncontrolled migration into the fillets (Morsy et al., 2014). Recently, the active coatings are the alternative way to eliminate these limitations, prolong the shelf life, improve safety, and enhance sensory properties of fish fillets.

Active packaging is a new concept in food application particularly in fish products. Chitosan polysaccharide is a unique coating substance in fish products because it is produced from shrimp, and due to its fishy taste, biocompatibility, high barrier, non-toxicty, and low cost (Yu, Regenstein, et al., 2018). Previous studies found that chitosan coatings have improved the quality of refrigerated fish fillets, *i.e.* grass carp (Ramezani et al., 2015), silver carp (Raeisi et al., 2016), yellow croaker (Wu et al., 2017), rainbow trout (Yu, Xu et al., 2018), and sea bass (Martínez et al., 2018). The natural extracts from herbs and spices have exhibited antioxidants (Baştürk et al., 2018)

*Correspondence to: mohamed.abdelhafez@fagr.bu.edu.eg

antimicrobials (Morsy et al., 2014), and flavored matters (Carney et al., 2018).

Green tea (*Camellia sinensis*) extract (GTE) is a natural substance widely used as a drink in Egypt, as well, it is rich in polyphenols and catechins that had healthy benefits, antimicrobial, and antioxidant properties (Carrizo et al., 2016). GTE is a Generally Recognized as Safe (GRAS) and classified by the European Union (EU) as a food additive (Randazzo et al., 2017). GTE has been incorporated in various food packages to extend shelf life based on inherent antioxidant properties (Muriel-Galet et al., 2015). In one study, extract of green tea was found to be effective against *Escherichia coli* (Roy et al., 2018). In another study, Noshad et al. (2017) incorporated green tea extract in mucilage coating in order to improve physicochemical and sensory properties of fried shrimps. De Lacey et al. (2014) demonstrated that green tea based films decreased the TVB-N and TMA-N, and extended the shelf life of hake fish.

Rosemary (*Rosmarinus officinalis* L) is one of the common herbs; its antioxidant properties are well recognized and approved by the EU (Moreno et al., 2006). RE is a good source of bioactive compounds including flavonoids, phenolics, diterpenoids, and triterpenes (Pineros-Hernandez et al., 2017). RE showed stronger inhibitory activity against *S. aureus, B. subtilis, E. coli,* and *S. typhium* (Mohsenabadi et al., 2018). *In vitro* study has demonstrated pullulan coating included rosemary was effective against *E. coli* O157:H7, *L. monocytogenes, S. aureus,* and *S. typhimurium* in meat products (Morsy et al., 2014). Also, the CMC coating enriched with rosemary extracts improved the oxidative stability in smoked eel fillets (Choulitoudi et al., 2017).

The aim of this work is to incorporate different antioxidant substances such as green tea (*Camellia sinensis*) and rosemary (*Rosmarinus officinalis* L.) extracts into chitosan coating on Tilapia fish fillets in order to retard the deterioration, lipid oxidation and microbial load during cold storage (4±1˚C).

Materials and Methods

Materials

Fresh Nile Tilapia (*Oreochromis niloticus*) fish weighted about (500±100g) were purchased in October 2017 from a local farm in Damietta Governorate, Egypt. The fish samples were transported in an isothermal ice-box to the Laboratory of Food Processing at Damietta University. The Tilapia fish composition was moisture 77.76%, protein 16.91%, crude fat 2.78%, and ash 0.99%. Green tea (*Camellia sinensis*) and rosemary (*Rosmarinus officinalis* L.) herbs were obtained from a local market in Cairo, Egypt. Chitosan was purchased from Technogen Company (Cairo, Egypt). Bacterial strains such as *Staphylococcus aureus* (ATCC 6538)*, Escherichia coli* (B-211)*, Bacillus cereus* (B-210)*,* and *Pseudomonas aeruginosa* (B-212) were supplied from Bacteriology Center, Faculty of Science, Assiut University.

Methods

Preparation of herbal extracts

The extraction of green tea and rosemary was done according to the method described by Skotti et al. (2014) with slight modifications. Briefly, the dry herbs were milled to fine powder using a Molineux grinder (MC300 Model, France). The powder was steeped in methyl alcohol 99% and then filtered through filter paper (Whatman NO. 4). The herbal extracts were concentrated with rotary evaporation at 50-60 ° C and then kept in cold storage until use.

Preparation of chitosan coatings

The coating solution of chitosan was prepared according to Abdallah et al. (2017) with some modifications. Briefly, 2% chitosan (*w/v*), 1% acetic acid (*v/v*), 1% Tween 80 (*w/v*), and 1.5% glycerol (*w/v*) were completely dissolved (~ 5 h). The chitosan solution was autoclaved for 15 min at 121˚C, then cooled to room temperature (25±1˚C). The green tea and/or rosemary extracts were added at (2 and 3%; *w/v*) and completely homogenized. The chitosan coatings were placed in refrigerator until use.

Fish fillets preparation and coating

The fish samples were prepared according to the steps as follows; beheaded, gutted, washed, and filleted (Cao et al., 2012). The fillet samples were divided into six groups: 1st group: green tea extract (GTE) 2%, 2nd group: green tea extract (GTE) 3%, 3rd: group rosemary extract (RE) 2%, 4th group: rosemary extract (RE) 3%, 5th group: chitosan alone (C^+), and the last one (Control; C). The fillet samples were immersed in chitosan coating for 5 min and repeated several times, then dried under the safety hood at room temperature (1hr). The coated fillets were packed and kept at 4±1˚C for 15 days. The fillet samples were analyzed at 0, 3, 6, 9, 12, and 15 days of storage time.

Determination of total phenolic compounds (TPCs)

The total phenolics in green tea and/or rosemary extracts were determined by the Folin-Ciocalteu spectrophotometric method (Li et al., 2007). The TPCs were expressed as mg of Gallic acid equivalents (GAE) per gram of the extract.

Determination of total flavonoid compounds (TFCs)

The total flavonoid compounds of green tea and/or rosemary extracts were estimated by a photometric method (Chang et al., 2002). The results of TFCs were expressed as mg quercetin equivalents (QE) per gram of the extract.

Determination of phenolic components using HPLC

The phenolic contents of green tea and rosemary were determined using HPLC (Hewlett-Packard -Model 1100) according to Mradu et al. (2012) with some modifications. The column C18 was the reversed phase (250×4.6 mm, 5µl particle size). The mobile phase contained acetic acid (0.5 %) in water at pH 2.6 (A), acetic acid (0.5%) in acetonitrile (B). The elution started with (A) and ended with (B) over 35 min, and the detector (UV) was 254 nm. The flow rate was 1 mL/min for total run of 70 min. Ten microliter of filtered sample were injected in Rheodyne injection valve (Model 7125) and the peaks were monitored at 280, 320, and 360 nm. The peaks were identified compared to retention times and UV-spectrum of the standards. All the standards were HPLC grade and from Sigma-Aldrich (St. Louis, MO, USA).

Radical scavenging activity of extracts using DPPH

The antioxidant activity of green tea and rosemary methanolic extracts was performed by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity according to Amarowicz et al. (2004). The DPPH scavenging capacity percentage was calculated. The IC_{50} value of extracts was calculated by a sigmoid non-linear regression model. The antiradical power (ARP) was calculated using ($1/IC_{50}$).

Determination of total volatile basic nitrogen (TVB-N)

The total volatile basic nitrogen (TVB-N) was determined according to the method of Egan et al. (1981). The values of TVB-N were expressed as mg N/100 g fish flesh.

Determination of thiobarbituric acid (TBA)

The thiobarbituric acid (TBA) was determined using spectrophotometr at 538 nm according to the method described by Vyncke (1970). The TBA values were expressed as mg malondialdehyde (MDA)/kg fish flesh.

pH value and water holding capacity (WHC)

The pH value was determined using a pH meter (model pH 211, HANNA instruments, Inc, USA). The WHC of fish samples were measured according to Egan et al. (1981).

Antibacterial activity of herbal extracts

Antibacterial activity of methanolic extracts of herbs was performed using well agar diffusion method according to Boyanova et al. (2005). The absence colonies on tested plates (zone inhibition) were measured and expressed as (millimeter).

Sensory evaluation

The Tilapia fish fillets were sensory evaluated during cold storage by ten member assessors from the Department of Food Industries, Damietta University. The fillets samples were prepared and put on plates coded with 3-digit numbers for assessment. The panelists were asked to evaluate fillets samples using 9-point hedonic scale for color, odor, and texture where; (9) like extremely, (8) like very much, (7) like moderately, (6) like slightly, (5) neither like nor dislike, (4) dislike slightly, (3) dislike moderately, (2) dislike very much, and (1) dislike extremely (Rong et al., 2009).

Statistical analysis

The statistical analyses of results were performed by one-way ANOVA with a significance level of $P \leq 0.05$ using the Co-STATE software. Data were analyzed as a completely randomized designs (Steel et al., 1997). Multiple comparisons test was carried out using a least significant difference (LSD) and Tukey's test.

Results and Discussions

Total phenolic, flavonoid compounds, and antioxidant activity of green tea and rosemary extracts

Total phenolic and flavonoid components have many benefits for human body like antimicrobial and antioxidant abilities. Data in Table 1 shows that the green tea extract (GTE) and rosemary extract (RE) had a high phenolic and flavonoid contents. The GTE has a higher content of phenolic compounds (156.51 mg as GAE g^{-1}) than RE (70.92 mg as GAE g^{-1}). Pereira et al. (2014) found that the content of phenolics in GTE

was 55.40 mg GAE g^{-1}. However, Afonso et al. (2013) reported that phenolics in RE was 16.67 mg GAE g^{-1}. This variance may be due to the species, method of extraction, and/or the type of solvent. Additionally, the current study shows a concentration of phenolic content is a higher than flavonoid content in GTE and RE. The obtained results are in agreement with reported by Pires et al. (2017).

TABLE 1. Total phenolic, total flavonoid contents and radical scavenging activity of green tea and rosemary extracts.

Extracts	Total phenolics (mg/g dw)	Total flavonoids (mg /g dw)	IC_{50} (µg/ml)	Anti-radical power (ARP)
Green tea extract (GTE)	156.51± 5.62	58.13± 2.11	17.93± 1.21	0.055 ± 0.002
Rosemary extract (RE)	70.92± 2.78	30.69± 1.55	74.56± 3.66	0.013± 0.00
Ascoribic acid (Why Ascorbic added here?)	-	-	8.38 ± 0.76	0.119± 0.01

Each value is represented as mean ± SD (n = 3).
Phenolics as Gallic acid equivalents; flavonoids as quercetin equivalents.

Moreover, Table 1 presents the antioxidant activity of GTE and RE (scavenge DPPH free radical). The GTE has a high free radical scavenging capacity (IC_{50}; 17.93 µg/mL), while RE has a low capacity (IC_{50}; 74.56 µg/mL) compared to ascorbic acid (IC_{50}; 8.38 µg/mL). It is noticed that there is an inverse relationship between the antioxidant activity and IC_{50} value. Bizuayehu et al. (2016) found that the GTE is rich in catechins and their derivatives as potential antioxidants. Satoh et al. (2016) demonstrated that IC_{50} value of GTE was low, while Xie et al. (2017) showed that IC_{50} of RE was high. The current results indicated that GTE and RE could be utilized as alternative substances of synthetic antioxidants. Furthermore, the antiradical power (ARP) of GTE was higher than RE. The antioxidant capacity of GTE and RE would be due to the phenolic and flavonoid compounds (Ali et al., 2015).

HPLC fingerprint of green tea and rosemary extracts

Data in Table 2 confirms that green tea and rosemary extracts exhibited the high amount of phenolic compounds. According to HPLC chromatograms (Fig. 1), twenty-three components were identified in GTE and RE. The major compounds in GTE having the following order Catechein > e-Vanillic > Chlorogenic > Pyrogallol > P-Hydroxy benzoic acid > Caffeine > Epicatechein > Vanillic. However, in RE the main compounds having the order as e-Vanillic > Salicylic > Benzoic > Catechein > Ellagic > Vanillic > Caffeine > Chlorogenic > Ferulic >. It was clear that in green tea extract, the Catechein represented about 796.9 mg/100g, while rosemary extract had e-Vanillic about 545.7 mg/100g. These results agreem with the results reported by Andrade et al. (2018).

Antimicrobial activity of green tea and rosemary extracts

The antimicrobial activity of GTE and RE at different concentrations, *i.e* 1, 2, 3 and 4% were examined against gram negative and gram-positive bacteria. Results in Table 3 demonstrated that GTE was more effective against of *Staphylococcus aureus*, while RE potently inhibited the growth of *Bacillus cereus and Staphylococcus aureus*. Whereas, all the concentrations of GTE and RE were not effective against *Escherichia coli* and *Pseudomonas aeruginosa*. These results agree with those reported by Hossain et al. (2014) who illustrated that green tea had antimicrobial activity against gram positive bacteria, however (Khalafalla et al., 2015) found that methanolic rosemary extract was not active against Pseudomonas Sp.

TABLE 2. Polyphenolic profile of green tea and rosemary extracts.

Phenolic compounds	Concentration(mg/100g)		
	Rt (min)	Green tea extract (GTE)	Rosemary extract (RE)
Pyrogallol	7.02	94.9	9.96
Gallic	7.11	15.5	5.87
4-Amino benzoic	7.39	16.4	5.17
Protocatechuic	8.37	37.9	6.48
Catechein	8.53	796.9	66.2
Catechol	9.10	9.39	6.29
Chlorogenic	9.17	157.6	39.5
Epicatechein	9.53	60.0	11.3
P-Hydroxy benzoic acid	9.67	73.5	15.4
Caffeine	9.88	65.5	40.7
Vanillic	10.11	53.8	41.6
Caffeic	10.17	12.3	1.27
P- coumaric	11.50	19.1	15.9
Ferulic	11.69	11.1	35.3
Iso-ferulic	12.10	8.47	25.4
e-Vanillic	12.98	209.7	545.7
Benzoic	13.07	11.7	95.1
Ellagic	13.21	49.4	56.6
α coumaric	13.28	ND	5.16
Coumarin	13.84	3.0	18.5
3,4,5- methoxy cinnamic	13.93	ND	5.63
Salicylic	14.10	29.1	266.9
Cinnamic	14.99	0.444	2.03

Rt = Retention time ND= Not detected

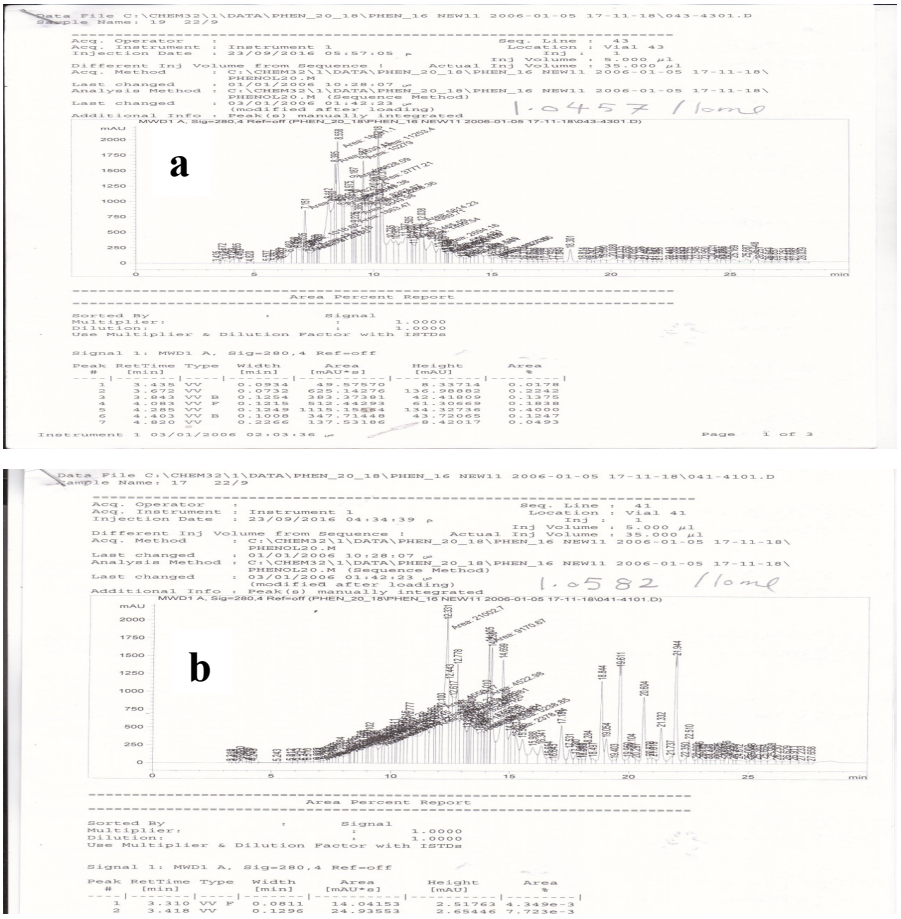

Fig.1.Titratable acidity% of fresh STPC as affected by using different ratios of BRF.

TABLE 3. Clear zones diameter (mm) of green tea and rosemary extracts against foodborne pathogens.

Microorganisms	Clear zones diameter (mm)							
	Green tea extract (GTE)				Rosemary extract (RE)			
	1%	2%	3%	4%	1%	2%	3%	4%
Bacillus cereus	8±1.11	8.5±1.22	10±1.26	11±1.02	13±1.28	14.5±1.33	16±1.60	16±1.25
Staphylococcus aureus	16±1.52	19±1.84	19.5±1.42	21±1.63	15±1.52	16±1.88	18±2.12	18.5±1.42
Escherichia coli	ND	ND	ND	ND	ND	ND	ND	ND
Pseudomonas aeruginosa	ND	ND	ND	ND	ND	ND	ND	ND

ND= Not detected

Impact of chitosan coating incorporation of green tea and rosemary extracts on lipid oxidation of fish fillets

The effect of green tea and rosemary extracts on malondialdehyde (MDA) content in fish fillet were shown in Fig. 2. The significant differences ($P \leq 0.05$) were observed in TBA between treated samples and the control sample. In general, the TBA value in fish fillet samples contained green tea extract (2 and 3%) and rosemary extract (2 and 3%) was significantly low compared to the control (without addition) during cold storage at 4 ºC. The TBA value in fillet samples ranged between 0.33 to 0.59 mg MDA/kg at zero time, then was increased rapidly in the control sample up to 0.92 mg MDA/kg at day 6th of storage, followed by (C^{+}; chitosan only) 0.89 mg MDA/kg at day 9th, then 2% GTE (0.73 mg MDA/kg) at day 12nd, 3% GTE (0.97 mg MDA/kg) at day 15th, 3% RE (0.90 mg MDA/kg) at day 12nd, and 2% RE (1.06 mg MDA/kg) at day 15th. The natural extracts from GTE or RE were decreased significantly ($P \leq 0.05$) TBA values even on the initial day of the storage. However, the lowering of lipid oxidation was highest in 2% GTE including fish sample as compared to other treatments. These results demonstrated that the use of extracts (GTE or RE) as an antioxidant source could be efficient in barring lipid oxidation of fish fillet at refrigerated storage (Pal et al., 2017).

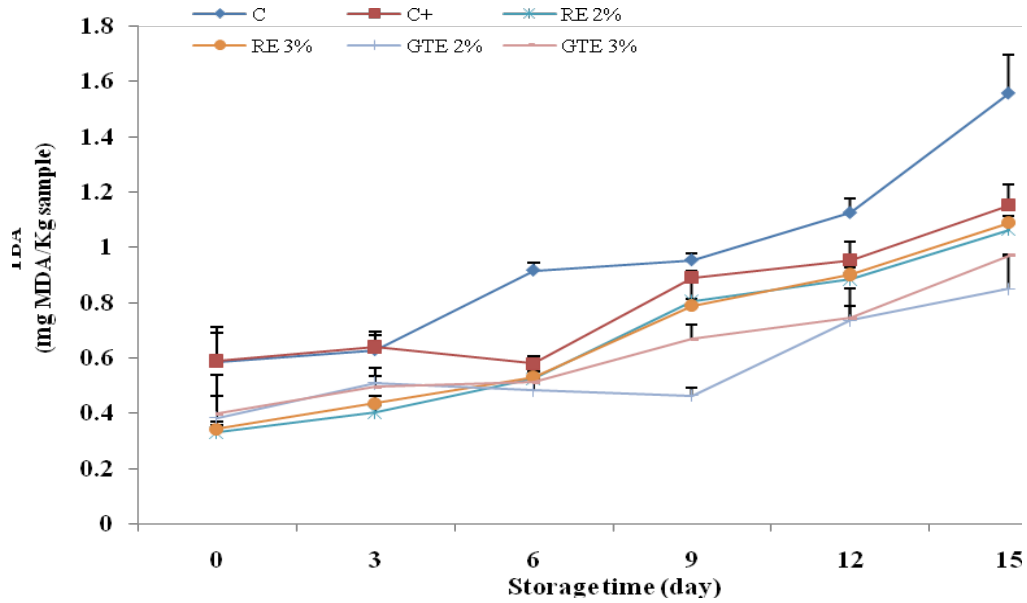

Fig.2. Effect of chitosan coating incroporation of green tea and rosemary extracts onTBA (mg MDA/Kg) in fish fillets during cold storage at (4°C). Error bars represent standard deviation, n= 3.

Impact of chitosan-based coating incorporation of green tea and rosemary extracts on protein degradation

The total volatile basic nitrogen (TVB-N) is one of the most biochemical parameters used to evaluate fish freshness (Saloko et al., 2014). TVB-N is commonly used as indicator of fish muscle deterioration and increase according to microbial and enzymatic spoilage. As presented in Fig. 3, the influence of green tea and rosemary extracts on TVB-N value in fish fillet during cold storage was evaluated. The herbal extracts and storage time had significant effects ($P \leq 0.05$) on protein quality. The TVB-N of control sample gradually increased from 14.53 to 78.86 mg N_2/100 g after 15 days of storage. The fillet treated with 2 and 3% rosemary extract showed the lowest value of TVB-N compared to green tea extract at the same concentration during cold storage. These results demonstrate the positive impact of rosemary extract on inhibition of microbial growth, mostly the proteolytic bacteria that cause a decomposition of protein to volatile compounds. These results agree with those reported by Feng et al. (2017).

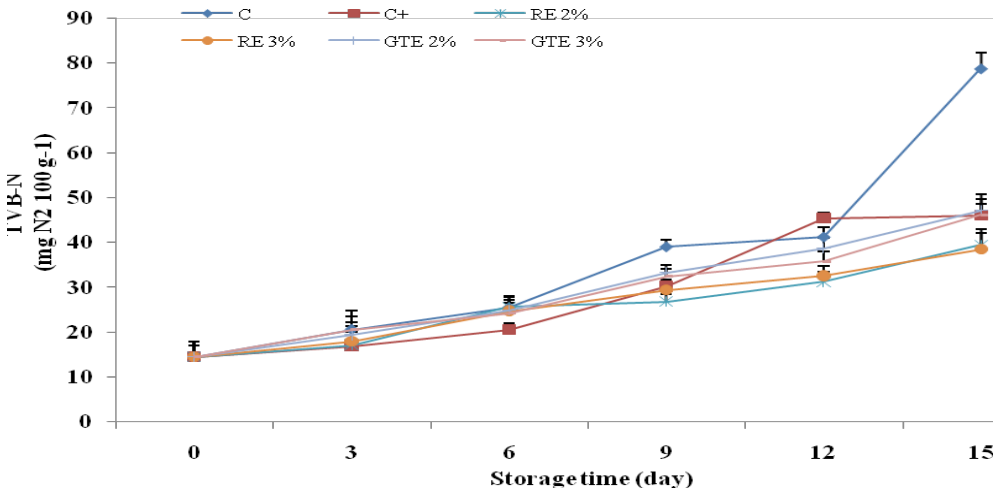

Fig. 3. Effect of chitosan coating incroporation of green tea and rosemary extracts onTVB-N (mg N_2 100 g^{-1}) in fish fillets during coldstorage at (4°C). Error bars represent standard deviation, n= 3.

Impact of chitosan coating incorporation of green tea and rosemary extracts on physical properties of fish fillets

Figure 4 illustrated the changes in pH value of fish fillets during cold storage at 4 ℃. There are no significant differences ($P \geq 0.05$) in pH value between fish fillet samples containing green tea and rosemary extracts compared to the control sample at zero time. However, after storage period, a significant difference ($P \leq 0.05$) was recorded between the treated samples. The pH value rapidly increased up to 6.95 after 9 days of cold storage in the control, while in fish samples including green tea and rosemary extracts at different concentrations showed a slight increase. The pH value of fillet samples containing 3% rosemary extract was 6.18, while in samples containing 2% green tea extract had a high pH value 7.04 after 15 days of cold storage. The increase of pH value in fish samples may be due to decomposition of the nitrogenous substances with endogenous or microbial enzymes (Li et al., 2017).

As shown in Fig. 5 the WHC of fish fillets coated with chitosan film including green tea and rosemary extracts was evaluated. The significant differences ($P \leq 0.05$) were recorded in WHC between the treated samples and the control during cold storage at 4˚C. The results indicated that higher WHC in fish fillet samples containing GTE or RE than the control. The GTE and/or RE addition at different concentrations in fish fillets improved the WHC value. The positive impact of GTE and/or RE chitosan-based coating on fillet samples might be attributed to the properties of GTE and/or RE as a water-binding substance as well chitosan film protect the samples from water loss. The results agree with those reported by Mohan et al. (2012).

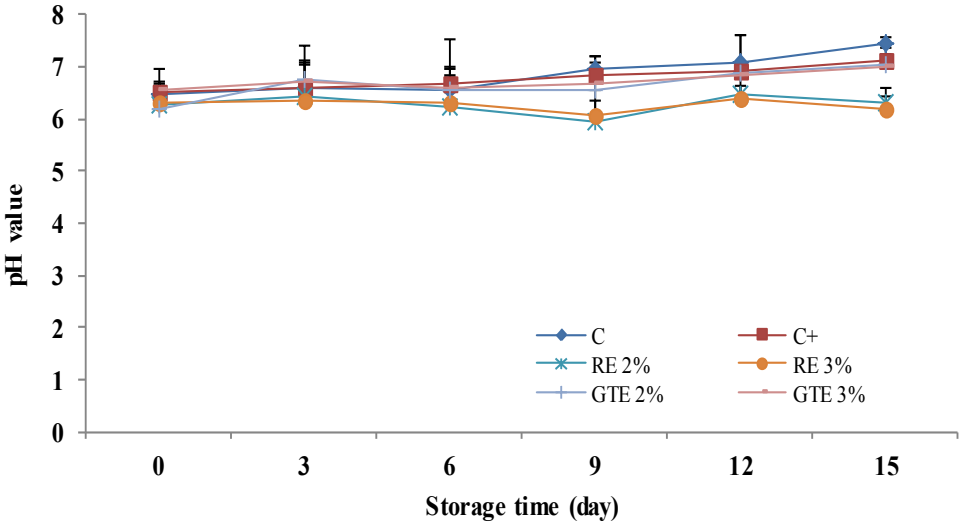

Fig. 4. Changes in the pH value in fish fillets with chitosan coating incroporation of green tea and rosemary extracts during cold storage at (4◦C).Error bars represent standard deviation, (n= 3).

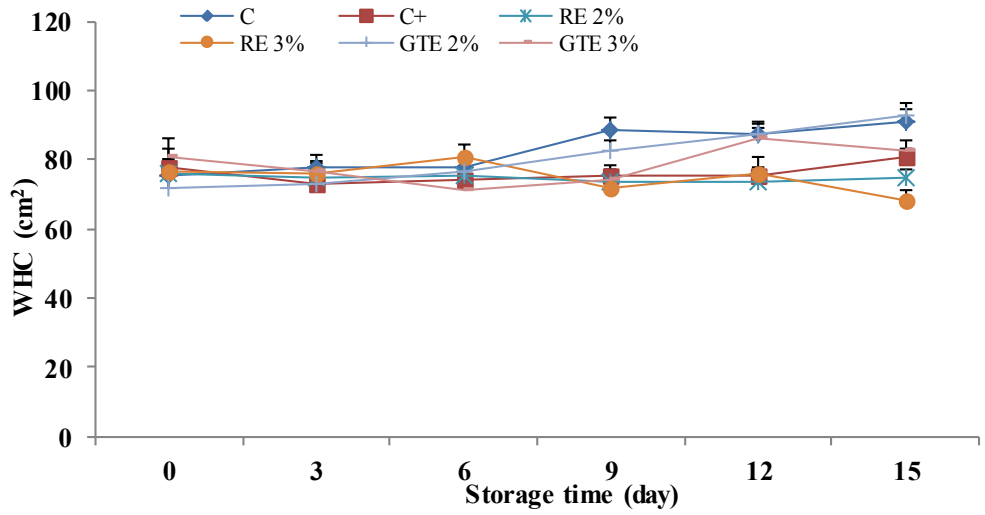

Fig.5. Effect of chitosan coating incroporation of green tea and rosemary extracts onWHC property in fish fillets during cold storage at (4◦C). Error bars represent standard deviation, (n= 3).

Impact of chitosan coating incorporation of green tea and rosemary extracts on microbial load of fish fillets

Figure 6 shows the TVBCs of Tilapia fillet samples coated with chitosan during refrigerated storage up to 15 days. A significant difference ($P \leq 0.05$) in TVBCs between the control and treated samples during the cold storage. In the control sample, the TVBCs of fillet were 3.69 $\log_{10}$ CFU/g at zero time then gradually increased up to 6.13 $\log_{10}$ CFU/g after 9 days. However, the samples containing 2% GTE, 3% GTE, 2% RE, and 3% RE exhibited counts of 5.12, 5.06, 4.57, and 4.58 $\log_{10}$ CFU/g, respectively. The addition of GTE and/or RE in fillets reduced the bacterial load significantly ($P \leq 0.05$) compared to the control. The results could be linked with those reported for TVB-N in (Fig. 4). This might be due to flavonoids and phenolic compounds in the GTE and RE, which have antimicrobial and antioxidant capacity (Tavassoli and Djomeh, 2011).

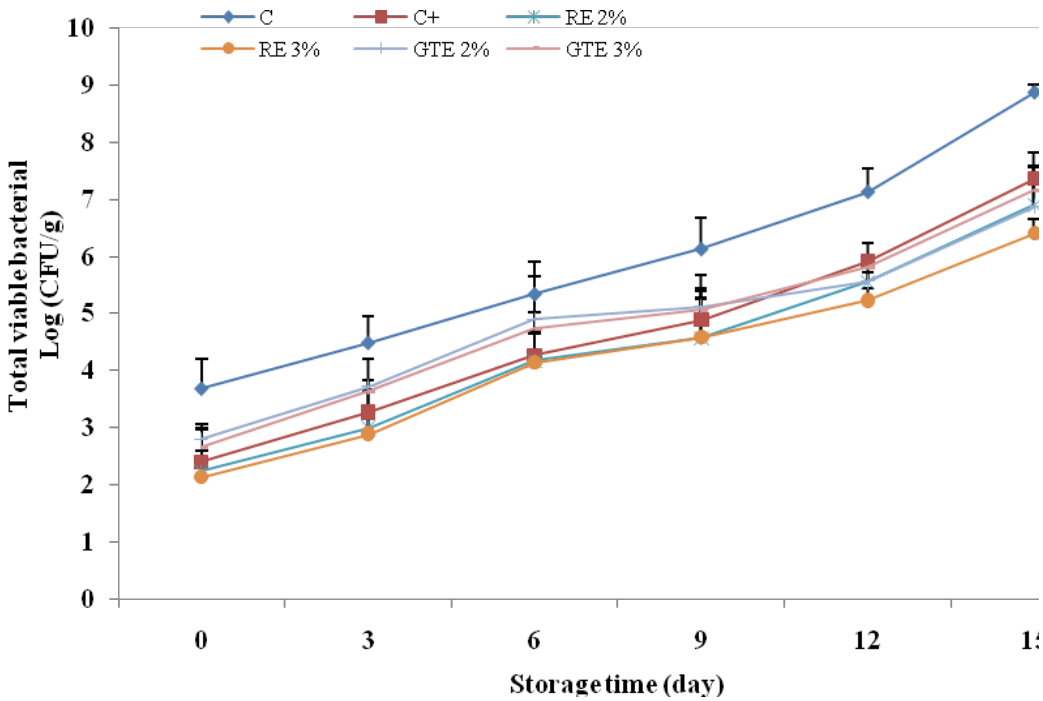

Fig.6. Effect of chitosan coating incroporation of green tea and rosemary extracts on total vaiable bacterial count in fish fillets cold storage at (4°C). Error bars represent standard deviation, (n= 3).

Sensory evaluation of fish fillets

The sensory attributes of the coated Tilapia fillets during cold storage were evaluated and shown in Fig 7. The sensory evaluation was performed up to day 6th in the control sample, day 9th in the samples coated with chitosan, and day 15th in the samples contained GTE or RE due to the off-odor that probably indicates decomposition and rejection (Gram et al., 2002). No significant differences ($P \geq 0.05$) were noticed between the treatments at zero time. The color score decreased gradually in most treatments with the increased storage time while no changes were noticed in the first day 9th in treated samples. The lowest color score was in the control sample while the samples contained GTE or RE were not affected. The changes in odor were increased in most treatments during the cold storage, which due to lipid oxidation and protein decomposition of fish fillets. The fillets samples contained GTE or RE were quite stable during the storage period while the changes in odor appeared obvious on day 15th. On the other hand, the control sample was a bit stable up to day 6th. Additionally, the fish fillet texture was affected with storage period. The significant differences ($P \leq 0.05$) were recorded between treated samples and the control. The samples contained GTE and or RE with different concentrations were accepted up to 15 days of storage, while the coated sample with chitosan was accepted until 9 days, and the control sample was rejected in day 9th. According to sensory evaluation and biochemical estimation, the shelf life of tilapia fillets extended up to 15 days in samples contained GTE or RE. As well as the changes in sensory parameters in the treated samples and the control had similar trends of TBA and TVB-N values. The results agree with those reported by Kostaki et al. (2009).

Conclusion

The methanolic extracts of green tea and rosemary exhibited a high amount of phenolic, antioxidant activity and antimicrobial properties. The activity of GTE and RE coatings is a promising approach to inhibit the protein and lipid oxidation in fish fillets. The GTE and/or RE improved the pH, WHC, and microbial quality of fillet samples during the storage time. Samples coated with GTE and/or RE were more sensory acceptable up to day 15th. The obtained results confirmed that GTE and/or RE coating successfully improved the quality and safety of Tilapia fish fillets and prolonging the shelf life.

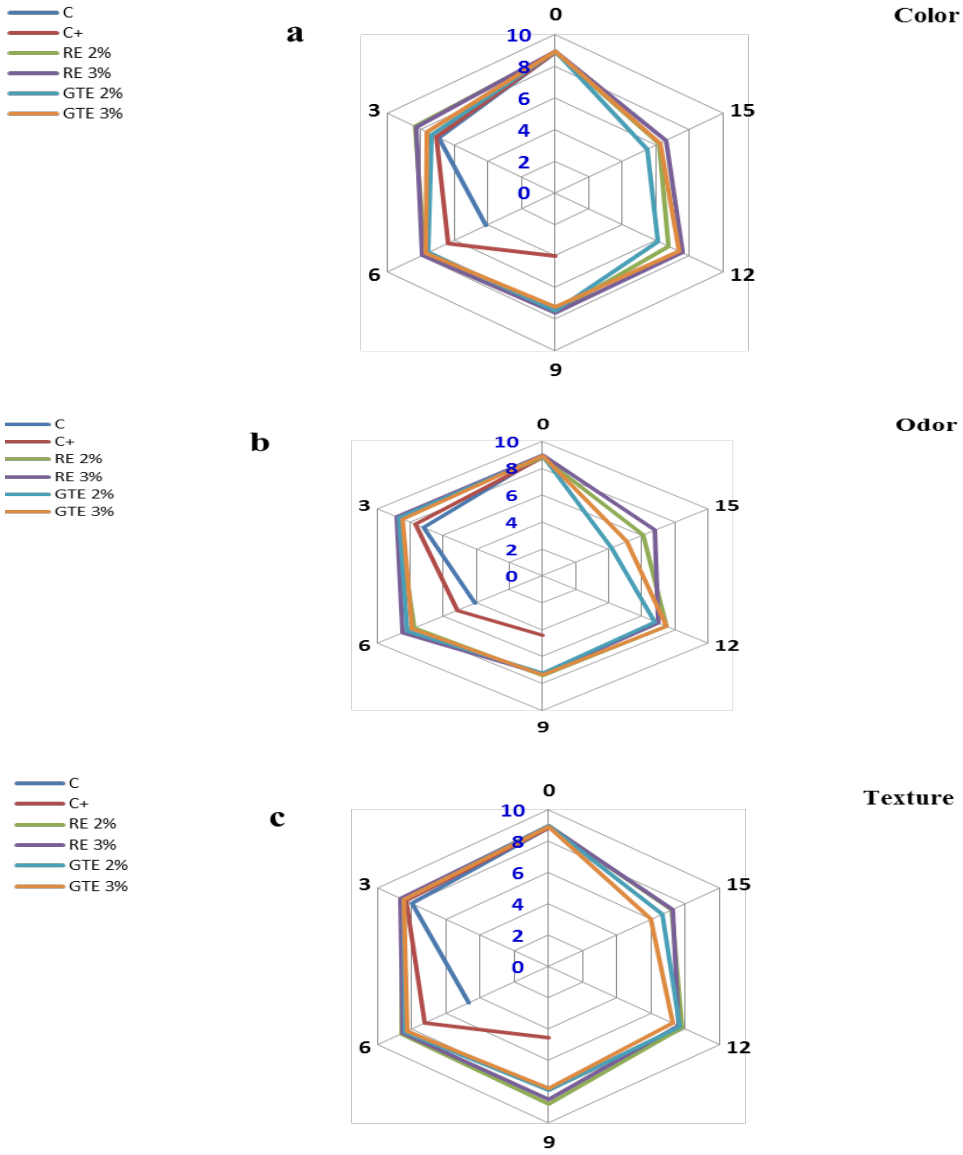

Fig. 7. Effect of chitosan coating incroporation of green tea and rosemary extracts on color (a), odor (b), and texture (c) in fish fillets during cold storage at (4°C).

References

Afonso, M.S., de O Silva, A.M., Carvalho, E.B., Rivelli, D.P., Barros, S.B., Rogero, M.M., Lottenberg, A.M., Torres, R.P. and Mancini-Filho, J. (2013) Phenolic compounds from Rosemary (*Rosmarinus officinalis L.*) attenuate oxidative stress and reduce blood cholesterol concentrations in diet-induced hypercholesterolemic rats. *Nutrition & Metabolism,* **10**, 19.

Ahmed, S., Ahmad, M., Swami, B.L. and Ikram, S. (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *Journal of Advanced Research,* **7,**17-28.

Ali, F.H., Hassan, A.-R.H. and Bahgat, A.E. (2015) Study of sumac extract (*Rhus coriaria L.*), lactic acid and thyme oil as decontaminants for shelf life extension of refrigerated rabbit meat. *Food Sci. Qual. Manag.* **38**, 71-78.

Amarowicz, R., Pegg, R., Rahimi-Moghaddam, P., Barl, B. and Weil, J. (2004) Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry,* **84**, 551-562.

Andrade, M.A., Ribeiro-Santos, R., Bonito, M.C.C., Saraiva, M. and Sanches-Silva, A. (2018) Characterization of rosemary and thyme extracts

for incorporation into a whey protein based film. *LWT,* **92**,497-508.

Baştürk, A., Ceylan, M.M., Çavuş, M., Boran, G. and Javidipour, I. (2018) Effects of some herbal extracts on oxidative stability of corn oil under accelerated oxidation conditions in comparison with some commonly used antioxidants. *LWT,* **89**,358-364.

Bizuayehu, D.; Atlabachew, M. and Ali, M.T. (2016) Determination of some selected secondary metabolites and their *in vitro* antioxidant activity in commercially available Ethiopian tea (*Camellia sinensis*). *Springer Plus,* 5, 412.

Boyanova, L., Gergova, G., Nikolov, R., Derejian, S., Lazarova, E., Katsarov, N., Mitov, I. and Krastev, Z. (2005). Activity of Bulgarian propolis against 94 *Helicobacter pylori* strains *in vitro* by agar-well diffusion, agar dilution and disc diffusion methods. *Journal of Medical Microbiology,* **54,** 481-483.

Cao, R., Liu, Q., Yin, B. and Wu, B. (2012) Chitosan extends the shelf-life of filleted tilapia (*Oreochromis niloticus*) during refrigerated storage. *Journal of Ocean University of China,* **11**,408-412.

Carney, E.M., Stein, W.M., Reigh, N.A., Gater, F.M., Bakke, A.J., Hayes, J.E. and Keller, K.L. (2018) Increasing flavor variety with herbs and spices improves relative vegetable intake in children who are propylthiouracil (PROP) tasters relative to nontasters. *Physiology & Behavior,* **188**, 48-57.

Carrizo, D., Taborda, G., Nerín, C. and Bosetti, O. (2016) Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. *Innovative Food Science & Emerging Technologies,* **33**, 534-541.

Chang, C.-C., Yang, M.-H., Wen, H.-M. and Chern, J.-C. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis,* **10**,178-182.

Chen, T.-Y., Chiou, T.-K., Ding, N.-F. and Pan, B.S. (2015) The impact of carbon-monoxide treatment on biochemical and sensorial quality *of Tilapia fillet during low temperature storage. Journal of Food and Nutrition Research,* **3**, 502-512.

De Lacey, A.L., López-Caballero, M. and Montero, P. (2014) Agar films containing green tea extract and probiotic bacteria for extending fish shelf-life. *LWT-Food Science and Technology,* **55**,559-564.

Egan, H., Kirk, R.S. and Sawyer, R. (1981) Pearson's chemical analysis of foods, Vol. 572. New York: Churchill Livingstone.

Feng, X., Ng, V.K., Mikš-Krajnik, M. and Yang, H. (2017). Effects of fish gelatin and tea polyphenol coating on the spoilage and degradation of myofibril in fish fillet during cold storage. *Food and Bioprocess Technology,* **10**, 89-102.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B. and Givskov, M. (2002) Food spoilage—interactions between food spoilage bacteria. *International Journal of Food Microbiology,* **78**,79-97.

Hossain, M.S., Nibir, Y.M., Zerin, S. and Ahsan, N. (2014) Antibacterial activities of the methanolic extract of Bangladeshi black tea against various human pathogens. *Dhaka University Journal of Pharmaceutical Sciences,* **13**, 97-103.

Khalafalla, F.A., Ali, F.H. and Hassan, A.-R.H. (2015) Quality improvement and shelf-life extension of refrigerated Nile Tilapia (*Oreochromis niloticus*) fillets using natural herbs. *Beni-Suef University Journal of Basic and Applied Sciences,* **4**, 33-40.

Ko, W.-C., Yang, S.-Y., Chang, C.-K. and Hsieh, C.-W. (2016) Effects of adjustable parallel high voltage electrostatic field on the freshness of tilapia (*Orechromis niloticus*) during refrigeration. *LWT-Food Science and Technology,* **66**, 151-157.

Kostaki, M., Giatrakou, V., Savvaidis, I.N. and Kontominas, M.G. (2009) Combined effect of MAP and thyme essential oil on the microbiological, chemical and sensory attributes of organically aquacultured sea bass (*Dicentrarchus labrax*) fillets. *Food Microbiology,* **26**, 475-482.

Li, H.-B., Cheng, K.-W., Wong, C.-C., Fan, K.-W., Chen, F. and Jiang, Y. (2007) Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry,* **102**, 771-776.

Li, Q., Zhang, L., Lu, H., Song, S. and Luo, Y. (2017). Comparison of postmortem changes in ATP-related compounds, protein degradation and endogenous enzyme activity of white muscle and dark muscle from common carp (*Cyprinus carpio*) stored at 4° C. *LWT-Food Science and Technology,* **78**, 317-324.

Liu, D., Liang, L., Xia, W., Regenstein, J.M. and Zhou, P. (2013) Biochemical and physical changes of grass carp (*Ctenopharyngodon idella*) fillets stored at− 3 and 0 C. *Food Chemistry,* **140**, 105-114.

Martínez, O., Salmerón, J., Epelde, L., Vicente, M.S. and de Vega, C. (2018) Quality enhancement of smoked sea bass (*Dicentrarchus labrax*) fillets by adding resveratrol and coating with chitosan and alginate edible films. *Food Control,* **85**, 168-176.

Mohan, C., Ravishankar, C., Lalitha, K. and Gopal, T.S. (2012) Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (*Sardinella longiceps*) during chilled storage. *Food Hydrocolloids,* **26**, 167-174.

Mohsenabadi, N., Rajaei, A., Tabatabaei, M. and Mohsenifar, A. (2018) Physical and antimicrobial properties of starch-carboxy methyl cellulose film containing rosemary essential oils encapsulated in chitosan nanogel. *International Journal of Biological Macromolecules,* **112**, 148-155.

Moreno, S., Scheyer, T., Romano, C.S.and Vojnov, A.A. (2006) Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research,* **40**, 223-231.

Morsy, M.K., Khalaf, H.H., Sharoba, A.M., El-Tanahi, H.H. and Cutter, C.N. (2014) Incorporation of essential oils and nanoparticles in pullulan films to control foodborne pathogens on meat and poultry products. *Journal of Food Science,* 79, 675-684.

Mradu, G., Saumyakanti, S., Sohini, M. and Arup, M. (2012) HPLC profiles of standard phenolic compounds present in medicinal plants. International *Journal of Pharmacognosy and Phytochemical Research,* **4**, 162-167.

Muriel-Galet, V., Cran, M.J., Bigger, S.W., Hernández-Muñoz, P. and Gavara, R. (2015) Antioxidant and antimicrobial properties of ethylene vinyl alcohol copolymer films based on the release of oregano essential oil and green tea extract components. *Journal of Food Engineering,* **149**, 9-16.

Noshad, M., Nasehi, B. and Anvar, A. (2017) Effect of active edible coating made by quince seed mucilage and green tea extract on quality of fried shrimps: physicochemical and sensory properties. *Nutrition and Food Sciences Research,* **4**, 31-36.

Pal, D., Chowdhury, S., Nath, S., Dora, K. and Majumder, R. (2017) Assessment of the shelf life of fish fillet treated with green tea extracts under refrigerated condition. *Biochemical and Cellular Archives,* **17**, 499-505.

Pereira, V., Knor, F., Vellosa, J. and Beltrame, F. (2014) Determination of phenolic compounds and antioxidant activity of green, black and white teas of *Camellia sinensis (*L.*)* Kuntze, *Theaceae. Revista Brasileira de Plantas Medicinais,* **16**, 490-498.

Pineros-Hernandez, D., Medina-Jaramillo, C., López-Córdoba, A. and Goyanes, S. (2017) Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging. *Food Hydrocolloids,* **63**, 488-495.

Pires, M.A., Munekata, P.E., Villanueva, N.D., Tonin, F.G., Baldin, J.C., Rocha, Y.J., Carvalho, L.T., Rodrigues, I. and Trindade, M.A. (2017) The antioxidant capacity of rosemary and green tea extracts to replace the carcinogenic antioxidant (BHA) in chicken burgers. *Journal of Food Quality,*1-6.

Raeisi, S., Sharifi-Rad, M., Quek, S.Y., Shabanpour, B. and Sharifi-Rad, J. (2016) Evaluation of antioxidant and antimicrobial effects of shallot (*Allium ascalonicum* L.) fruit and ajwain (*Trachyspermum ammi (*L.) *Sprague*) seed extracts in semi-fried coated rainbow trout (*Oncorhynchus mykiss*) fillets for shelf-life extension. *LWT-Food Science and Technology,* **65**, 112-121.

Ramezani, Z., Zarei, M. and Raminnejad, N. (2015) Comparing the effectiveness of chitosan and nanochitosan coatings on the quality of refrigerated silver carp fillets. *Food Control,* **51**, 43-48.

Randazzo, W., Falcó, I., Aznar, R. and Sánchez, G. (2017) Effect of green tea extract on enteric viruses and its application as natural sanitizer. *Food Microbiology,* **66,** 150-156.

Rong, C., Chang-hu, X., Qi, L. and Bang-zhong, Y. (2009) Microbiological, chemical and sensory assessment of (I) whole ungutted,(II) whole gutted and (III) filleted tilapia (*Oreochromis niloticus*) during refrigerated storage. *International Journal of Food Science & Technology,* **44**, 2243-2248.

Roy, N., Mandal, S., Mahanti, B. and Dasgupta, S. (2018) Antimicrobial activity of Green Tea: A comparative study with different Green Tea extract. *PharmaTutor,* **6**, 23-29.

Saloko, S., Darmadji, P., Setiaji, B. and Pranoto, Y. (2014) Antioxidative and antimicrobial activities of liquid smoke nanocapsules using chitosan and maltodextrin and its application on tuna fish preservation. *Food Bioscience,* **7,** 71-79.

Satoh, T., Fujisawa, H., Nakamura, A., Takahashi, N. and Watanabe, K. (2016) Inhibitory effects of eight green tea catechins on cytochrome P450 1A2, 2C9,

2D6, and 3A4 activities. *Journal of Pharmacy & Pharmaceutical Sciences,* **19,** 188-197.

Skotti, E., Anastasaki, E., Kanellou, G., Polissiou, M. and Tarantilis, P.A. (2014) Total phenolic content, antioxidant activity and toxicity of aqueous extracts from selected Greek medicinal and aromatic plants. *Industrial Crops and Products,* **53,** 46-54.

Steel, R., Torrie, J. and Dickey, D. (1997) Principles and procedures of statistics: a biometrical approach. 3rd ed. New York, N.Y.: McGraw-Hill.

Tavassoli, S. and Djomeh, Z.E. (2011 Total phenols, antioxidant potential and antimicrobial activity of methanol extract of rosemary (*Rosmarinus officinalis* L.). *Global Veterinaria,* **7,** 337-341.

Tveteras, R.; Nystoyl, R. and Jory, D. (2018) Global fish production estimates & trends. Global Aquaculture Alliance, 1-62.

Vyncke, W. (1970) Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. *European Journal of Lipid Science and Technology,* **72,** 1084-1087.

Wang, F., Zhang, H., Jin, W. and Li, L. (2018) Effects of tartary buckwheat polysaccharide combined with nisin edible coating on the storage quality of tilapia (*Oreochromis niloticus*) fillets. *Journal of the Science of Food and Agriculture,* **98,** 2880-2888.

Wu, T., Wu, C., Fang, Z., Ma, X., Chen, S. and Hu, Y. (2017) Effect of chitosan microcapsules loaded with nisin on the preservation of small yellow croaker. *Food Control,* **79,** 317-324.

Xie, J., VanAlstyne, P., Uhlir, A. and Yang, X. (2017) A review on rosemary as a natural antioxidation solution. *European Journal of Lipid Science and Technology,* **119,** 1600439.

Yu, D., Regenstein, J.M. and Xia, W. (2018) Bio-based edible coatings for the preservation of fishery products: A review. *Critical Reviews in Food Science and Nutrition,* 1-46.

Yu, D., Xu, Y., Regenstein, J.M., Xia, W., Yang, F., Jiang, Q. and Wang, B. (2018) The effects of edible chitosan-based coatings on flavor quality of raw grass carp (*Ctenopharyngodon idellus*) fillets during refrigerated storage. *Food Chemistry,* **242,** 412-420.

(*Received:*22/10/2018;
*accepted:*30/10/2018)

تأثير أغلفة الشيتوزان المحتوية على مستخلصات الشاي الأخضر والروزماري علي خصائص الجودة الفيزيوكيميائية والميكروبية لشرائح سمك البلطي المخزنة بالتبريد

زينب صبري فرج[١] ، أشرف محمد شرف[١] و محمد خيري مرسى[٢]
[١] قسم الصناعات الغذائية، كلية الزراعة، جامعة دمياط , مصر
[٢] قسم الصناعات الغذائية، كلية الزراعة، جامعة بنها , مصر

تعتبر تقنية الأغلفة الحيوية المحتوية على مستخلصات طبيعية من التقنيات المبتكرة في الآونة الأخيرة، حيث تلعب دوراً جوهرياً فى تحسين جودة وسلامة منتجات الأسماك. إستهدفت هذه الدراسة تقييم مدى فعالية أغلفة الشيتوزان المحتوية على مستخلصات الشاي الأخضر والروزماري ضد أربعة أنواع من البكتريا الممرضة الاستافيلوكوكس اويرس، الاشريشيا كولاى، باسلس سيريس، وزيدوموناس اوريجونوزا، كذلك أيضاً تأثير أغلفة الشيتوزان على الخصائص الفيزيائية والبيوكيميائية لشرائح سمك البلطي خلال التخزين بالتبريد على درجة حراره 4±1م° لمدة ١٥ يوم. خلال هذه الدراسه تم تقييم مستخلصي الشاي الأخضر والروزماري بتركيز 2 و 3% ضد سلالات البكتريا الممرضة مقارنة بعينة الشيتوزان بدون إضافة مستخلصات طبيعية وعينة الكنترول (بدون إضافة الشيتوزان). أيضاً تم تطبيق أغلفة الشيتوزان المحتوية على مستخلصى الشاي الأخضر والروزماري على شرائح سمك البلطى وأمكن تقييم الخواص الفيزيائية (الأس الهيدروجيني , القدرة علي مسك الماء), مؤشرات الطزاجة (حمض الثيوبارابيوتريك ، القواعد النيتروجينية الكلية المتطايره), الحمل الميكروبي (العد الكلى للبكتريا)، والتقييم الحسي (اللون, الرائحة والقوام). أوضحت النتائج أن مستخلصي الشاي الأخضر والروزماري ذات محتوى مرتفع من المركبات الفينولية وذات كفاءة عالية كمضاد للاكسدة. أيضاً أشارت النتائج إلى إنخفاض قيم حمض الثيوبارابيوتريك ، القواعد النيتروجينية الكلية المتطايره بينما حدث إرتفاع فى القدرة على مسك الماء فى شرائح السمك المحتوية على مستخلصي الشاي الأخضر والروزماري مقارنةً بعينة (الكنترول) خلال فترة التخزين بالتبريد (١٥ يوم). لوحظ أيضاً إنخفاض الحمل الميكروبي في العينات المعاملة بمستخلصي الشاي الأخضر والروزماري مقارنة بعينة الكنترول. كما أظهرت نتائج التقييم الحسي لصفات (اللون, الرائحة والقوام) أن العينات المعاملة كانت مقبولة حسياً حتى نهاية فترة التخزين بالتبريد (١٥ يوم). لذلك، وطبقاً للنتائج المتحصل عليها يوصى بإستخدام أغلفة الشيتوزان المحتوية على المستخلصات الطبيعية (الشاي الأخضر والروزماري) على شرائح منتجات الأسماك نظراً لأنها تؤدى إلى منع تحلل البروتين، تأخير تزنخ الدهون، خفض الحمل الميكروبى وإطالة فترة الصلاحية لشرائح سمك البلطي.