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Quality enhancement in refrigerated tiger tooth croaker (*Otolithes ruber*) fillets using chitosan coating containing green tea extract

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Abstract

The effects of chitosan (2%) coating combined with green tea extract (GTE) (1%) on the quality of tiger-tooth croaker (*Otolithes ruber*) fillets including chemical, microbiological, texture, color and sensory properties during 16 days of refrigerated storage was investigated. The color changes were significantly retared, and the texture parameteres and sensory scores were significantly improved in tiger- tooth croaker coated by chitosan, GTE and chitosan combined with GTE, compared with the control. The coincidental lowered rate of increase total volatile base (TVB) content, thiobarbitoric acid (TBA), peroxide value (POV) and free fatty acids (FFA) were obtained in tiger-tooth croaker coated chitosan+GTE. Chitosan in combination with GTE had higher inhibition on microbial growth and yielded the tigertooth croaker with higher likeness, comapared with the other treatments. Therefore, fish coated with chitosan+GTE had the lowest losses in quality during refrigerated storage.

Keywords: Tiger-tooth croaker, Chitosan coating, Green tea extract, Quality

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Introduction

The tiger-tooth croaker (*Otolithes ruber*; Sciaenidae), also known as "Shurideh" in Persian, is one of the most important fish in Iran with the highest economic value. Croakers are mainly offered in the Iranian market as skinned and boneless fillets. Major changes occur in proximate, microbiological, chemical and sensory composition of fish fillets during storage in the refrigerator (Sharifian *et al*., 2011). Because the tiger-tooth croaker is consumed domestically and exported in large quantities, it is very important to extend its shelf life of this fish during chilled storage. There has been some research to determine the shelf life of *O. ruber* during chilled storage (Sharifian *et al*., 2011; Ninan and Zynudheen, 2014).

Chitosan, a linear polysaccharide of randomly distributed β- (1-4)- linked D-glucosamine and N-acetyl- D-glucosamine, is a biocompatible polysaccharide obtained from deacetylation of chitin. Edible coating is a thin layer of edible material formed as a coating on a food because of their structural properties (Falguera *et al*., 2011). In the food industry, Chitosan coatings have been used successfully because they have some advantages such as edibility, biodegradability, aesthetic appearance and barrier properties, being nontoxic and non-polluting, as well as carrier of foods additives (i.e. antioxidants, antimicrobials). Therefore, theses coatings can retain quality of raw, frozen and processed foods including fish items by preventing bacterial growth and delaying lipid oxidation (Gniewosz *et al*., 2014).

Interest in edible coatings and plant extract on highly perishable seafood has intensified in recent years (Li *et al*., 2013; Yuan *et al*., 2016). To preserve the fish fillet, antioxidant additives prior to packaging is a common method has been used in food market to extend the shelf life of aquatic products. Currently, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) frequently have been used prior to packaging. However, recent tendency is that consumers' demand fillets with natural preservatives like green tea extract (GTE), usually packaged in edible materials which can reinforce the mechanical properties of coating (Siripatrawan and Harte, 2010). Green tea is a good source of polyphenolic compounds such as catechina having strong antioxidant and antimicrobial properties and radical scavenging activities (Cabrera *et al*., 2006). Catechines of green tea extract are comprised for four compounds which are epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallat. Incorporating GTE as a food additive due to antioxidant activities is a growing interest in the seafood industry (Lin and Lin, 2005; Nirmal and Benjakul, 2011). GTE can improve the marketing potential of various seafood products and can effectively be used in the packaging food industry. Several authors have demonstrated that some of the compounds present in green tea extracts possess antibacterial properties (Perumall and Hettiarachchy,

2011). Numerous researchers have reported the effectiveness of green tea extract for retarding the oxidation of unsaturated fatty acids in various seafood productions (Lin and Lin, 2005; Nirmal and Benjakul, 2011).

Oxidation of lipids and growth of microorganisms in seafood products is considered as one of the main factor limiting product quality and acceptability (Frankel, 1993). Antioxidants and antibacterial activities of GTE in combined with edible coating refers to the prevention or delay the onset of lipids oxidation by inhibiting the initiation or propagation step of the oxidative chain reactions or forming stable radicals (Huang *et al*., 2005), metal chelation and single oxygen quenching (Mastromatteo *et al*., 2010) and so decreasing of microbial load.

Recently, natural preservation like green tea extract has been used as the natural safe additives in the food industry (Li *et al*., 2013). The use of green tea extract treatment and edible coating might retard the quality loss of seafood production stored in refrigerator. Therefore, the aim of this study was to investigate the combinative effect of GTE and chitosan coating on the shelf life of *O. ruber* fillets by monitoring microbiological, chemical and sensory changes throughout the storage at refrigerated temperature during 16 days.

Materials and methods

Fish preparation

O. ruber with an average weight of 520 g were caught with gill net in the Persian Gulf, Khorramshahr, Iran in July 2015. Fish were placed in crushed ice with a fish/ice ratio 1:3 (w/w) and transported to the fish processing laboratory with 2-3 hrs. after catching. They were washed with tap water and two fillets were obtained from each fish after removing the head and gutted.

Preparation of green tea extracts (GTE)

GTE was prepared according to the method of Nirmal and Benjakul (2011). Green tea powder was treated with chloroform using a powder/ solvent ratio of 1:20 (w/v) to remove chlorophyll. The mixture was stirred for 30 min and then filtered through Whatman No.1 filter paper. To prepare GTE, the de-chlorophilsed green tea powder (2 g) was mixed with 80 ml of 80% ethanol at 40 °C for 2 hrs with continuous stirring. The extract was filtered through a Whatman filter paper No.1. The filtrate was concentrated by a rotary evaporator (IKA RV 05 basic, Germany). The concentrated samples were dried in hot air oven at 60 °C for 12 hrs. GTE powder was kept in a polyethylene bag and placed in a desiccator in dark at 4 °C until use.

Preparation of coating solution and treated fillets

Chitosan solution was prepared with 2% (w/v) chitosan (Sigma Chemical Co., medium molecular weight, viscosity 200- 800 cp) in 1% (v/v) acetic acid (Nowzari *et al*., 2013). To achieve complete dispersion of chitosan, the solution was stirred at 40 ºC to dissolve completely for 3 hrs.

Glycerol was added at 0.75 ml/g concentration as a plasticizer and stirred for 30 min. Then the green tea aqueous extract solution was mixed with chitosan solution for 30 min to obtain the final concentrations of 1% (w/v) of green tea in the chitosan solution. The resulting solution was homogenized using homogenizer. All samples were stored at 4 °C. Each sample were randomly taken and used as sample for proximate composition, microbiological and chemical analyses every 4 days up to 16 days.

Proximate composition analyses

Moisture, protein, fat and ash contents of samples were measured in triplicate according to AOAC (1984).

Microbiological analyses

The samples (25 g) were placed in a stomacher bag containing 225 ml of 0.85% saline water. After mixing for 1 min in a stomacher blender, further serial dilution was done using the same diluent. Thereafter, 0.1 ml of appropriate dilution was used for microbiological analysis by spread plate method. The media and condition used were: Plate Count Agar (PCA, Merck, Denmark, Germany) incubated for psychrotrophic bacteria count at 4 °C for 10 days and for total aerobic plate count at 30 °C for 24-48 h (Sallam, 2007).

Chemical analyses

Total volatile basic nitrogen value was estimated by the micro- diffusion method (Goulas and Kontominas, 2005). Peroxide value of fish muscle was measured according to the Woyewoda *et al*. (1986) method. Thiobarbituric acid measurement was determined following the method of Siripatrawan and Noipha (2012). The free fatty acids values were determined in the lipid extract by the procedures of Woyewoda *et al.* (1986).

Texture measurements

Textural profile analysis (TPA) were performed using an LFRA-4500 texture analyzer (Brookfield Engineering Laboratories, inc., Middleboro, MA) equipped with a 4.5-kg load cell and texture Pro Lite V1.0 software. Samples removed from the dorsal part of the fish of size 3.5×3.5×0.7 cm were compressed perpendicularly using a 20-mm diameter cylindrical probe. The testing conditions were two consecutive cycles at 25% compression, cross- head movement at a constant speed of 0.8 mm s^{-1}, and a trigger point of 22.5g. Texture variables (hardness and springiness) were calculated as described by Kilinc *et al*. (2009), and Hernandez *et al*. (2009).

Color measurements

A Minolta Chroma Meter CR400 (Minolta, Osaka, Japan) was used for color measurements. Colors were expressed as CIELab coordinates. In this system, $\mathbf{L^*}$ represents the color lightness on a 0-100 point scale from black to white; $\mathbf{a^*}$ is the position between red (+) and green (-); and $\mathbf{b^*}$ is the position between yellow (+) and blue (-). The color intensity is expressed by a chroma value ($\mathbf{C^*_{ab}}$), while hue ($\mathbf{H^0_{ab}}$) corresponds to the name of the color as found in its pure

state on the spectrum. These values were calculated according to the formulae:

$C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$ and $H^0_{ab} = \arctan(b^*/a^*)$

Sensory evaluation

Samples were prepared by steaming for 60 min at 80 °C. Salt (1.5%) was added. The cooked samples were evaluated by 10 panelists from the Department of seafood processing with the ages of 23-28 (7 females and 3 males), using the 5-point hedonic scales where 5: like extremely; 3: neither like or nor dislike; 1: dislike extremely. Panelists were regular consumers of fish and had no allergies to fish. All panelists were asked to evaluate for odor and flavor (Ojagh *et al*., 2010).

Statistical analyses

All experiments were performed in triplicate and a completely randomized design were used. Analysis of variance (ANOVA) was performed and mean comparisons were done by Duncan's multiple range tests. For pair comparison, T-test was used. Analysis was performed using a SPSS package (SPSS 11.0 for windows, SPSS Inc, Chicago, II, USA). P values less than 0.05 were considered statistically significant.

Results

The mean compositional contents of moisture, protein, lipid, and ash in the tiger-tooth croaker fillet analyzed were 76.45±0.14%, 14.05±0.97%, 4.58±0.22%%, and 2.08±0.03%, respectively.

Variations in the value of total viable counts (TVC) and psychrotrophilic bacteria (PTC) of fish treated without and with chitosan solution containing GTE during the refrigerated storage are presented in Table 1. The initial TVC in the control tiger-tooth croaker fillet was 2.66 $\log_{10}$ CFU g^{-1}, it was 2.33 $\log_{10}$ CFU g^{-1} for other treatments. The PTC in the control, chitosan, GTE and chitosan+ GTE samples were 2.00, 1.66, 2.66 and 2.00 $\log_{10}$ CFU g^{-1}, respectively, which were indicated of high quality and proper manufacturing practices.

Table 1 depicted the variation of TVB-N value of tiger-tooth croaker during the storage. The initial TVB-N of samples varied from 8.73 mg N 100^{-1} to 10 mg N $100g^{-1}$ muscle. The TVB-N level increased gradually along with the storage time in all samples (p<0.05), but the increasing rate varied with treatments. When fish treated with GTE with or without chitosan, the lower TVBN level was observed, compared to the control samples (p<0.05). At day 16, samples treated with GTE+chitosan showed the lowest TVB content (p<0.05). TVB content increased rapidly and reached 36.40 mg N $100g^{-1}$ muscle after 16 days of storage. However, all samples stored under GTE and chitosan had TVB content less than 25 mg N $100g^{-1}$ muscle within 16 days of storage.

TBA values of fish stored with coating chitosan (with or without GTE) during refrigerated storage are presented in Table 1. At 0 day, TBA value of all samples was found between 0.59 and 0.83 mg malonaldehyde kg^{-1}

muscle. TBA value of all samples increased when the storage time increased (p<0.05).

Table 1: Compared effects of chitosan and green tea extract on the changes of total viable count (TVC) and psychrotrophic count (PTC) of tiger tooth croaker during refrigerated storage.

Days of storage		0	4	8	12	16
TVC (log10cfu g^{-1})	Control	2.66±0.88^{cA}	4.66±0.88^{cA}	6.00±0.57^{cA}	9.66±1.20^{bA}	13.33 ±1.76^{aA}
	Chitosan	2.33±0.66^{bA}	2.33±0.88^{bAB}	3.66±0.88^{abAB}	6.00±0.57^{aB}	5.66±1.45^{aB}
	GTE	2.33±0.88^{aA}	3.00±0.57^{aAB}	3.66±1.20^{aAB}	5.00±0.57^{aBC}	4.33±1.20^{aB}
	Chitosan+GTE	2.33±0.33^{aA}	1.66±0.33^{aB}	3.00±0.57^{aB}	2.66±0.33^{aC}	3.00±0.57^{aB}
PTC (log10cfu g^{-1})	Control	2.00±0.57^{dA}	4.00±0.57^{dA}	6.66±0.33^{cA}	10.00±1.15^{bA}	12.33 ±0.33^{aA}
	Chitosan	1.66±0.33^{cA}	2.66±0.66^{cA}	3.00±0.57^{bcB}	6.00±0.57^{abB}	6.00±1.73^{aB}
	GTE	2.66±0.33^{bA}	3.00±0.57^{abA}	3.66±0.88^{abB}	5.66±0.88^{aB}	5.00±1.15^{abB}
	Chitosan+GTE	2.00±0.57^{aA}	2.33±0.88^{aA}	3.33±0.88^{aB}	4.66±1.20^{aB}	4.00±1.15^{aB}

Different letters in the same row within the same treatment indicate the significant differences (p<0.05). Different capital letters in the same column within the same storage time indicate significant differences (p<0.05). Values are means ± standard error (n=3).

Concentrations of primary oxidation products in the lipid fraction of the fillets measured as POV during the 16 days storage, are presented in Fig.3. The initial POV ranged from 0.53 to 0.90 meq peroxide 1000g lipid. In general, POV value of all samples increased up to day 12, then decreased on day 16 (Table 2), so the highest score was on 12th for control samples (2.88 meq peroxide 1000g^{-1} lipid). Both the primary and secondary oxidation products have been assessed to consider the complexity of the lipid oxidation process. The initial FFA value was from 1.50 to 2.26% of oleic acid. A gradual increase in FFA formation in all samples was observed, but chitosan+GTE fillets, decelerated developing process of FFA production during storage of refrigerated (Table 1). Color values including **L*** coordinate (lightness indicator), **a*** value (indicator of tendency toward the red for **a*** negative) and **b*** value (indicator of the tendency toward yellow for **b*** negative) of control tiger-tooth croaker samples and those coated with chitosan and GTE during storage 4 $^{\circ}$C are shown in Table 3. Storage time had a significant effect on all the color parameters. In general, the variation in color of fish samples were in white to yellowish color, which was illustrated by **L*** value> 37, **a*** value ranging from -0.62 to -1.28, and **b*** value ranging from -0.86 to -3.51.

Table 2: Chemical changes of fish samples during refrigerated storage.

Days of storage		0	4	8	12	16
TVBN (mg N 100g^{-1} muscle)	Control	8.73±1.10^{eA}	15.73±1.81^{dA}	21.00±1.61^{cA}	25.66±0.46^{bA}	36.40 ±0.80^{aA}
	Chitosan	9.66±1.26^{bA}	11.26 ±0.46^{bB}	17.26±0.46^{aB}	17.26±0.46^{aB}	19.60±0.80^{aB}
	GTE	10.00±0.80^{cA}	11.26±0.46^{cB}	14.93±0.93^{bBC}	16.80±1.40^{bB}	21.00±0.80^{aB}
	Chitosan+GTE	9.53±1.26^{bA}	10.00±1.55^{bB}	12.93±0.54^{abC}	14.93±1.68^{aB}	15.60±1.62^{aC}
TBA (mg MDA KG^{-1} muscle)	Control	0.84±0.04^{cA}	1.26±0.09^{cA}	3.40±0.23^{bA}	4.15±0.44^{bA}	8.22±0.24^{aA}
	Chitosan	0.59±0.11^{cA}	0.81±0.09^{bcB}	1.48±0.13^{bcB}	2.08±0.22^{bB}	3.81±0.81^{aB}
	GTE	0.83±0.07^{bA}	0.95±0.03^{bB}	1.24±0.17^{bB}	1.24±0.11^{bC}	4.32±0.69^{aB}
	Chitosan+GTE	0.59±0.03^{bA}	0.84±0.03^{bB}	1.06±0.01^{bB}	1.20±0.01^{bC}	2.40±0.55^{aB}
POV (meq peroxide 1000g^{-1} lipid)	Control	0.53±0.15^{cA}	1.41±0.03^{bA}	1.83±0.09^{bA}	2.69±0.18^{aA}	2.88±0.10^{aA}
	Chitosan	0.67±0.11^{dA}	1.04±0.09^{cB}	1.24±0.13^{bcB}	1.97±0.04^{aB}	1.40±0.12^{bB}
	GTE	0.78±0.10^{bA}	1.18±0.04^{bB}	1.70±0.22^{aAB}	1.82±0.21^{aB}	1.00±0.05^{bB}
	Chitosan+GTE	0.90±0.04^{cA}	1.08±0.02^{bcB}	1.37±0.07^{abAB}	1.60±0.21^{aB}	1.47±0.14^{abB}
FFA (% oleic acid)	Control	1.50±0.30^{dA}	3.86±0.24^{cA}	3.93±0.20^{bA}	7.36±0.53^{bA}	10.74±0.95^{aA}
	Chitosan	1.60±0.30^{dA}	2.40 ±0.26^{cB}	3.09±0.22^{cB}	4.09±0.21^{bB}	5.45±0.16^{aB}
	GTE	1.67±0.25^{cA}	2.18±0.26^{cB}	3.14±0.35^{bAB}	3.96±0.23^{bB}	5.10±0.29^{aBC}
	Chitosan+GTE	2.26±0.42^{cA}	1.91±0.17^{bcB}	2.37±0.11^{bcB}	2.82±0.25^{abC}	3.44±0.23^{aC}

Different letters in the same row within the same treatment indicate the significant differences (p<0.05). Different capital letters in the same column within the same storage time indicate significant differences (p<0.05). Values are means ± standard error (n=3).

Table 3: Compared effects of chitosan and green tea extract on the changes of color of tiger tooth croaker during refrigerated storage.

Different letters in the same row within the same treatment indicate the significant differences (p<0.05).

Days of storage		0	4	8	12	16
L*	Control	58.99±1.19^{aA}	49.43±0.51^{bB}	45.51±0.77^{cD}	41.01±0.72^{dB}	37.37±0.51^{eD}
	Chitosan	58.99±1.19^{aA}	51.30±0.54^{bB}	51.28±0.45^{bC}	42.37±0.61^{cB}	40.53±0.20^{cC}
	GTE	58.99±1.19^{aA}	55.41±1.10^{bA}	52.93±0.25^{bB}	46.70±1.25^{cA}	43.42±0.69^{dB}
	Chitosan+GTE	58.99±1.19^{aA}	56.48±0.52^{bA}	55.28±0.28^{bA}	48.42±1.17^{cA}	45.10±0.40^{dA}
a*	Control	-1.28±1.19^{bA}	-0.82±0.03^{aA}	-0.50±0.21^{aA}	-0.75±0.01^{aA}	-0.62±0.00^{aA}
	Chitosan	-1.28±1.19^{cA}	-0.91±0.00^{aB}	-1.01±0.01^{bB}	-0.92±0.00^{aB}	-0.88±0.02^{aAB}
	GTE	-1.28±1.19^{cA}	-1.03±0.00^{bC}	-0.96±0.03^{bB}	-0.88±0.00^{aB}	-0.87±0.00^{aB}
	Chitosan+GTE	-1.28±1.19^{cA}	-1.07±0.01^{bC}	-0.95±0.01^{aB}	-0.92±0.00^{aB}	-0.92±0.00^{aC}
b*	Control	-3.51±0.09^{dA}	-3.14±0.01^{cA}	-2.26±0.06^{bA}	-2.13±0.01^{bA}	-0.86±0.06^{aA}
	Chitosan	-3.51±0.09^{dA}	-3.26±0.04^{dAB}	-2.86±0.06^{cB}	-2.54±0.07^{bB}	-1.66±0.12^{aB}
	GTE	-3.51±0.09^{cA}	-3.38±0.08^{cBC}	-2.91±0.13^{bBC}	-2.62±0.04^{abB}	-2.38±0.04^{aC}
	Chitosan+GTE	-3.51±0.09^{cA}	-3.51±0.06^{cC}	-3.18±0.06^{bC}	-2.98±0.02^{bC}	-2.57±0.17^{aC}
C$^{*}_{ab}$	Control	3.73±0.09^{aA}	3.24±0.01^{bC}	2.37±0.06^{cB}	2.24±0.01^{cC}	1.06±0.05^{dC}
	Chitosan	3.73±0.09^{aA}	3.38±0.04^{bBC}	3.03±0.05^{cA}	2.69±0.06^{dB}	1.88±0.11^{eB}
	GTE	3.73±0.09^{aA}	3.53±0.08^{aAB}	3.06±0.13^{bA}	2.76±0.03^{cB}	2.49±0.04^{cA}
	Chitosan+GTE	3.73±0.09^{aA}	3.67±0.06^{aA}	3.32±0.06^{bA}	3.12±0.02^{bA}	2.72±0.15^{cA}
H$^{0}_{ab}$	Control	1.21±0.00^{bA}	1.31±0.01^{aA}	1.25±0.00^{bAB}	1.22±0.00^{bB}	0.94±0.03^{cC}
	Chitosan	1.21±0.00^{bA}	1.29±0.00^{aA}	1.22±0.01^{bC}	1.21±0.00^{bB}	1.07±0.02^{cB}
	GTE	1.21±0.00^{cA}	1.26±0.00^{aB}	1.24±0.00^{bBC}	1.23±0.00^{bB}	1.20±0.00^{cA}
	Chitosan+GTE	1.21±0.00^{bA}	1.27±0.00^{aB}	1.27±0.00^{aA}	1.26±0.00^{aA}	1.21±0.02^{bA}

Different capital letters in the same column within the same storage time indicate significant differences (p<0.05). Values are means ± standard error (n=3).

In general, the hardness of tiger-tooth croaker in all five treatments was significantly decreased during refrigerated storage (Table 4). However, the hardness of fish treated by GTE, chitosan coating and chitosan

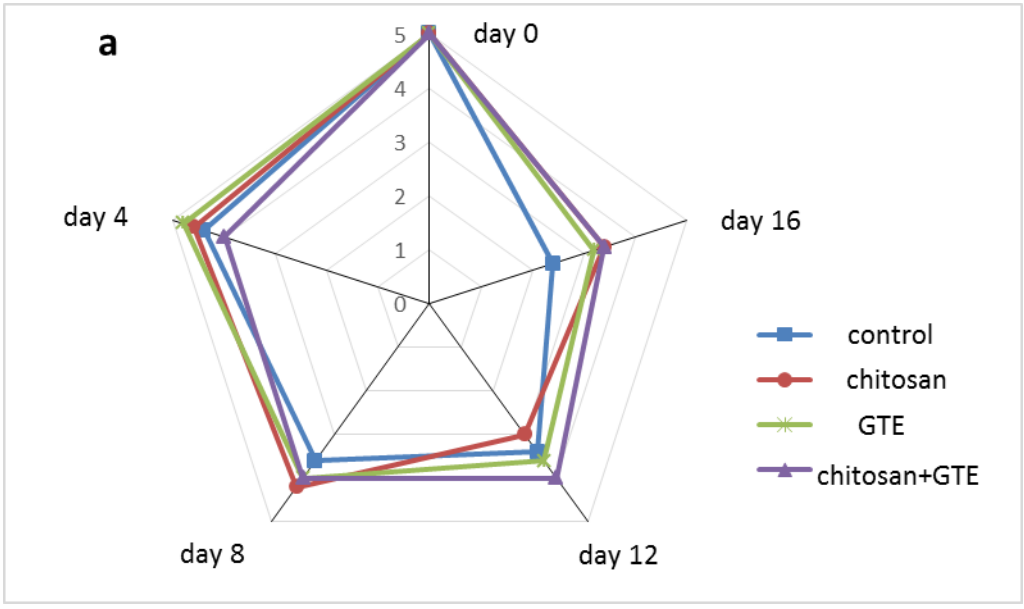
coating+GTE was significantly improved (p<0.05) as compared with the control. The hardness of chitosan coating+GTE showed lower changes than control samples.

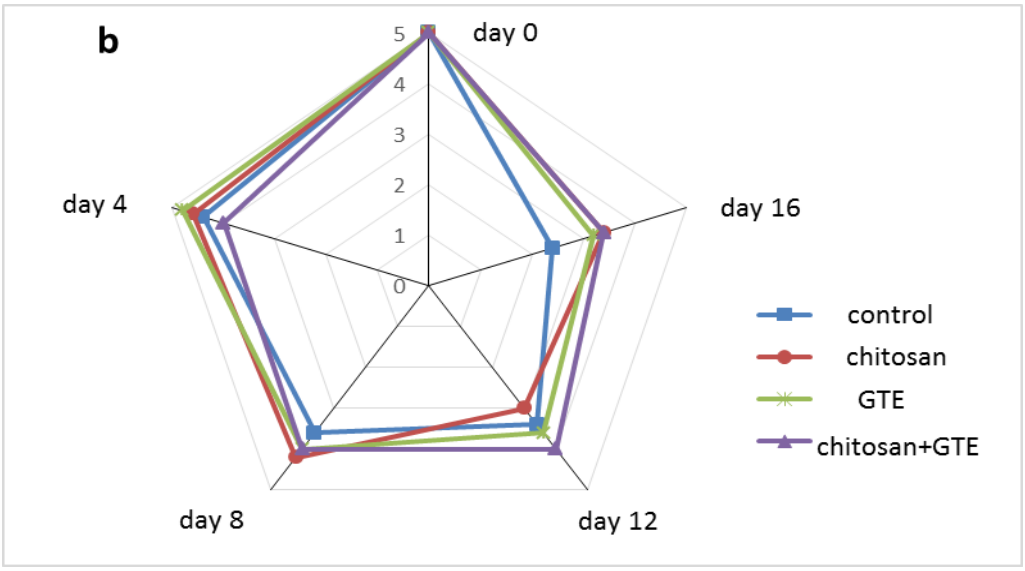
Table 4: Compared effect of chitosan and green tea extract on the changes of texture of tigertooth croaker during refrigerated storage.

Days of storage		0	4	8	12	16
Hardness (gf)	Control	926.61±9.10^{aA}	812.33±1.47^{bC}	730.27±13.47^{cC}	653.57±6.76^{dB}	543.52±13.34^{eC}
	Chitosan	926.61±9.10^{aA}	846.69±4.77^{bB}	723.49±4.73^{cC}	680.44±19.01^{cdB}	666.11±21.48^{dB}
	GTE	926.61±9.10^{aA}	871.51±10.11^{bA}	788.84±8.90^{cB}	797.07±2.97^{cA}	718.06±5.00^{dA}
	Chitosan+GTE	926.61±9.10^{aA}	83.84±3.78^{bA}	821.63±6.09^{cA}	808.95±2.69^{cA}	738.22±11.06^{dA}
Springiness (mm)	Control	2.25±0.04^{aA}	2.13±0.01^{bB}	1.41±0.04^{cC}	1.21±0.02^{dC}	1.15±0.01^{dB}
	Chitosan	2.25±0.04^{aA}	2.18±0.01^{aAB}	1.62±0.05^{bB}	1.33±0.03^{cB}	1.22±0.02^{cB}
	GTE	2.25±0.04^{aA}	2.20±0.01^{aA}	1.78±0.02^{bA}	1.51±0.02^{cA}	1.39±0.04^{dA}
	Chitosan+GTE	2.25±0.04^{aA}	2.23±0.01^{aA}	1.86±0.05^{bA}	1.55±0.03^{cA}	1.36±0.04^{dA}

Different letters in the same row within the same treatment indicate the significant differences (p<0.05). Different capital letters in the same column within the same storage time indicate significant differences (p<0.05). Values are means ± standard error (n=3).

Means of sensory attributes scores including texture, odor, color, flavor and overall acceptance of control samples and those coated with chitosan, GTE and chitosan+ GTE during refrigerated storage are shown Fig. 1. At day 0, no differences in likeness were found between all treatments. These results suggested that treatment of tiger-tooth croaker with GTE with prior chlorophyll removal had no impact on color and flavor of treated fish. At the end of storage, the decreased in likeness for all attributes were observed for all samples.

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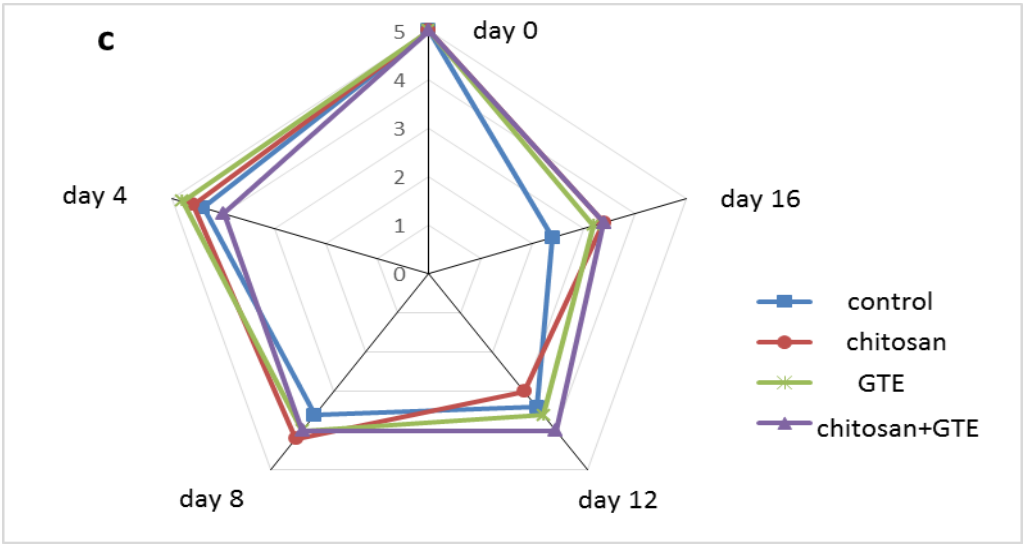
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control
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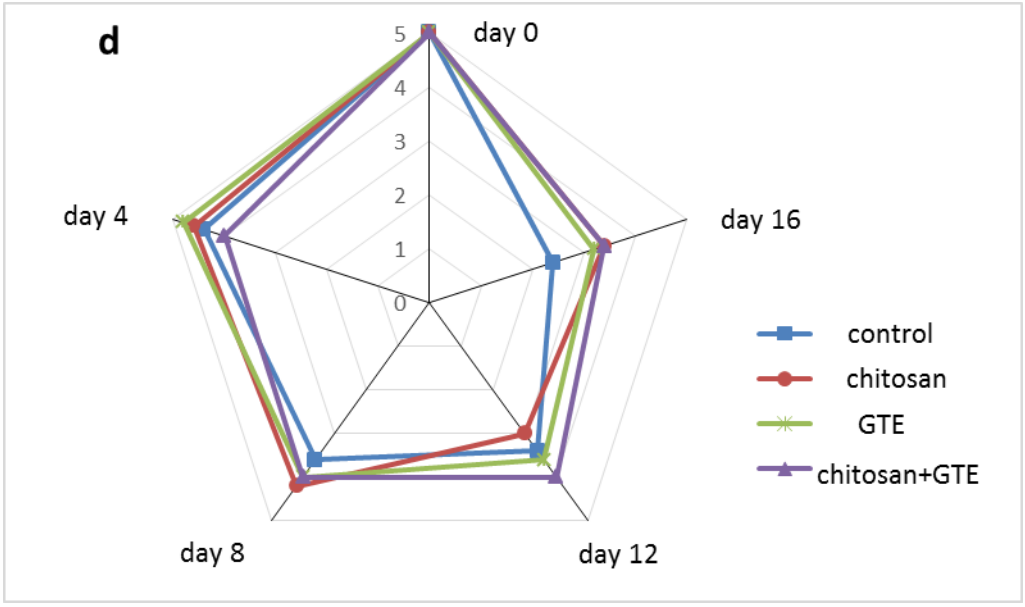
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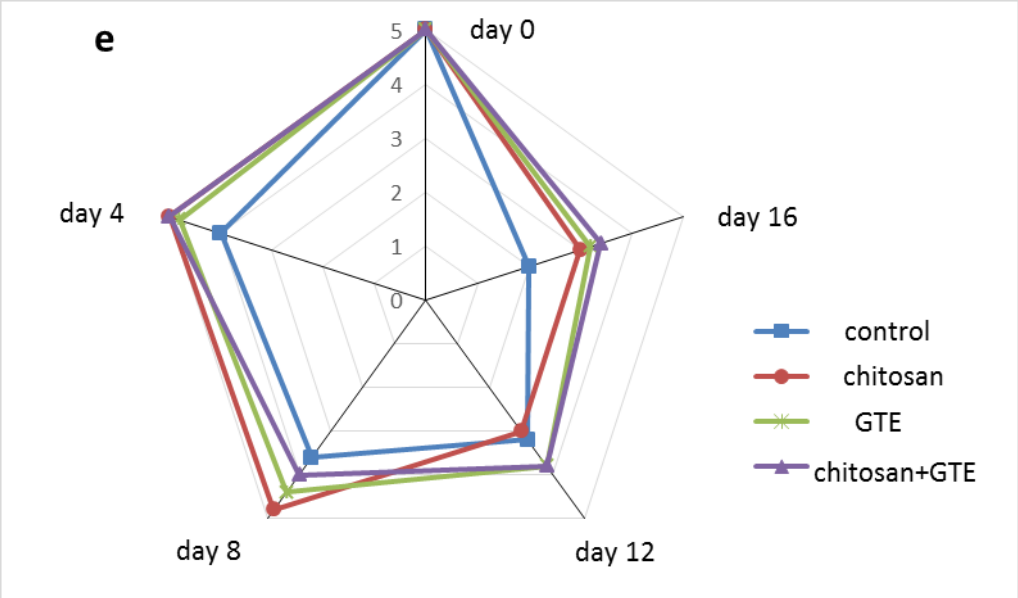

Figure 1: Compared effects of chitosan and green tea extract on the changes of sensory of tiger tooth croaker during refrigerated storage. (a) texture; (b) odor; (c) color; (d) flavor; (e) overall acceptance scores.

Discussion

Ninan and Zynudheen (2014) and Sharifian *et al*. (2011) showed some differences in the proximate composition of the tiger-tooth croaker fillets especially for the lipids content. Such variations in the proximate composition of fish depend on the nutrition, catching season (spawning cycles), sexual variation, fish size, living area, feeding pattern as well as the other environmental conditions.

The gram-negative psychrophilic bacteria are important agents of spoilage stored seafood at chilled temperatures (Gram and Huss, 1996; Sallam, 2007). In general, TVC and PTC of the control increased continuously as the storage time increased (p<0.05). Increasing in TVC was lowered when fish were coated with chitosan+GTE. The retardation of microbial growth of coated samples with chitosan+GTE was mainly attributed to the immediate antimicrobial effects of chitosan solution and GTE (Perumall and Hettiarachchy, 2011). Although the antimicrobial effects of GTE were inferior to chitosan`s, it is noteworthy that the lowest microbial count among all three treatments and also the control were those of samples containing both chitosan and GTE, indicating a synergistic effects, in agreement with the finding of Yuan *et al*. (2016) for fresh Pacific white shrimp during refrigerated storage. A microbiological acceptability limit is 7 log CFU g^{-1} for fresh water and marine species that is fit for human consumption (ICMSF, 1986). By the day 12 of storage, control samples exceeded the value of 7 log CFU g^{-1} for TVC and PTC, which was regarded as the upper acceptability limit for raw fish (ICMSF, 1986) while that of all treatments did not achieve this count at the end of 16 days storage

time. Several studies have shown that chitosan and GTE are effective as antimicrobial agents and can improve fresh fish quality (Li *et al*., 2013; Yuan *et al*., 2016). The antimicrobial effects of chitosan are through to be related to the presence of the positively charged on the NH_3^+ group of glucosamine monomer in chitosan molecules which interact with negatively charged macromolecules on the microbial cell surface, leading to the leakage of intracellular constituents of the microorganisms moreover, the mechanism of action of chitosan appears to be related to disruption of the lipopolysaccharide layer of the outer membrane of gram-negative bacteria (Pereda *et al*., 2011), also to its function as a barrier against oxygen transfer (Jeon *et al*., 2002). Incorporation of chitosan coating with GTE increased the antimicrobial properties of the coating, as TVC and PTC in tiger-tooth croaker coated with chitosan+GTE were lower than coated with control. Lorenzo *et al*. (2014) reported that green tea extract decreased bacterial growth in pork patties also Siripatrawan and Noipha (2012) found that green tea extract led to a decrease of total viable counts in pork sausages. Nirmal and Benjakul (2011) reported that green tea extract could inhibit psychrotropic bacteria, enterobacteriaceae and H_2S-producing bacteria growth effectively during 10 days of refrigerated storage. The increase of antimicrobial activities of chitosan+GTE is potentially attributed to green tea polyphenols and catchines by inhibition of DNA and RNA synthesis of bacteria cells (Mori *et al*., 1987), inhibition of outer cell membrane or cytoplasmic membrane function of bacteria (Tsuchiya et al., 1994), interfering with energy metabolisms of bacteria (Haraguchi *et al*., 1998), reinforced the oxygen barrier of coatings, and thus decreased bacterial growth.

TVB usually includes calculation of trimethylamine, dimethylamine, ammonia and other volatile bases, which impart characteristic off-flavors to fish (Goulas and Kontominas, 2007). TVB`s are products of spoilage bacterial such as *S. putrefaciens* and *P. phosphoreum*, autolytic and endogenous enzymes, which used as index to assess the keeping quality and shelf life of seafood products (Etemadian *et al*., 2012) A level of 25 mgN $100g^{-1}$ muscle has been considered the highest acceptable level (Kilincceker *et al*., 2009) and above 25-30 mgN $100g^{-1}$ muscle indicates that fish are decomposed and inedible (Etemadian *et al*., 2012). For all samples except control sample, TVBN level was less 25 mgN 100g muscle, indicating the fillets of fish maintained at a good quality during storage. According to these results, it was found that using a coating of chitosan combined with GTE resulted in a more rapidly reduced bacterial population or decreased capacity of bacteria or both (Li *et al*., 2013). Polyphenols presents in GTE have demonstrated inhibitory effects on bacteria. The antimicrobial activities of GTE when combined with bacteriocins like chitosan have demonstrated more effectiveness, when

they have been used alone against bacteria to oxidative deamination of non- protein nitrogen compounds (Perumalla and Hettiarachchy, 2011). Li *et al*. (2013) observed higher amounts of TVB-N for un-coated rainbow trout fillets without natural preservations at 20th day of refrigerated storage. This finding may be attributed to the increased antimicrobial activity of chitosan combined with GTE induced reduction in the capacity of bacteria to perform oxidative deamination of non- protein nitrogen compounds (Li *et al*., 2013).

The increase in TBA during storage may be attributed to the partial dehydration of fish and interacting between lipids with air oxygen (Kilincceker *et al*., 2009). Lower increase in TBA value was observed in chitosan+ GTE coated samples. This observation was similar to the results of Li *et al*. (2013). Siripatrawan and Noipha (2012) reported the effectiveness of chitosan coating and GTE and found lower contents of TBA in chitosan coated+GTE pork sausage samples compared to the un-coated samples throughout the storage time. This result suggested that oxidation of lipids in fish samples could be minimized by using of chitosan coating probably due to the antioxidant activity as well as its low oxygen permeability of chitosan. The antioxidant mechanism of chitosan could be by chelate action of ion metals and/or the combination with meat lipids during storage (López-Caballero *et al*., 2005). Incorporation of GTE into chitosan coating enhanced the antioxidant properties of chitosan, as TBA values of GTE+chitosan coated samples were lower than those of the un-coated samples, which are effective of free radicals scavengers and metal chelation (Siripatrawan and Noipha, 2012). The antioxidant activities of the inferred GTE polyphenols (mainly flavanoids) can exhibit scavenging activity against free radicals, superoxide radicals, peroxynitrite chelate copper and ion, preventing metal catalyzed free radical formation (Farhoosh *et al*., 2007). TBA value of 5 mg malonaldehyde kg^{-1} muscle is an acceptable limit, while the fish may be consumed up to the level of 8 mg malonaldehyde kg^{-1} (Sallam, 2007). In the current study, TBA value for control, chitosan, GTE and chitosan+ GTE was 8.22, 3.81, 4.32 and 2.40 mg malonaldehyde kg^{-1} sample, respectively, at the end of the storage.

The highest score was for 12^{th} day of control samples (2.88 meq peroxide $1000g^{-1}$ lipid). This is probably because of the decomposition of peroxide to secondary products (i.e.: aldehydes) or their interaction with muscle proteins (Jeon *et al*., 2002) (Table 2). At the end of the storage POVs of control, chitosan, GTE and chitosan+GTE was 2.88, 1.40, 1.00 and 1.47 meq peroxide $1000g^{-1}$ lipid, respectively. All of coatings and GTE showed less POV than control, it means that chitosan coated with GTE could decrease lipid oxidation of fillets. Kamil *et al*. (2002) had been reported that chitosan may be considered as a potential natural antioxidant for stabilizing lipids containing foods. Incorporating of GTE with chitosan increased the antioxidant

properties (Siripatrawan and Noipha, 2012). These results are in agreement with Ojagh *et al*. (2010), who reported that chitosan coating was effective in retarding the production of primary lipid oxidation products in trout fillets stored at refrigeration (4±1˚C). In present study, coating with or without GTE was more effective than control samples to retarding POV of fillets (p<0.05).

Chitosan+GTE fillets decelerated developing process of FFA production during storage of refrigerated (Table 1) due to the action of lipase and phospholipase on phospholipids and triglycerides (Rostamzad *et al*., 2011). FFA value of control samples was higher than treated samples, significantly (p<0.05). At the end of the storage FFA value was10.74, 5.45, 5.10 and 344 % of oleic acid for control, chitosan, GTE and chitosan+GTE, respectively. As it was concluded from POV and TBA values, chitosan+GTE coatings protect fish fillets so would reduce production of free fatty acids.

The appearance of muscle is the major value for acceptability and preference, which influenced by both muscle structure characteristics and pigments concentrations (Ginés *et al*., 2004). The coating treatments, with or without GTE corporation, affected the $\mathbf{L^*}$, $\mathbf{a^*}$, $\mathbf{b^*}$, $\mathbf{C^*_{ab}}$, and $\mathbf{H^0_{ab}}$ values of fish samples storage at refrigerated. Control samples showed significantly lower values of $\mathbf{L^*}$, $\mathbf{C^*_{ab}}$ and $\mathbf{H^0_{ab}}$ values during storage than samples coated with chitosan and GTE samples. Color loss in fish fillets during storage might be attributed to the lipids oxidation, oxidation of proteins with haemo groups (haemoglobin and myoglobin), non- enzymatic browning reactions between lipids oxidation products and the amine groups in proteins, and microbial spoilage (Siripatrawan and Noipha, 2012). Samples coated with chitosan showed lower changes in color values, probably due to the antioxidant and antimicrobial properties of the chitosan. Polyphenolic compounds of green tea extract, including EC, ECG, EGCG, C, GCG, CG, and GC can also help improve the color stability of fresh fish products (Siripatrawan and Noipha, 2012; Namal Senanayake, 2013). Studies conducted by Siripatrawan and Noipha (2012) have been shown that the pork sausages improved by the addition of chitosan and GTE compared to the control samples. The results suggested that incorporation GTE into chitosan coating could increase the antioxidant and antimicrobial properties of the chitosan.

Texture of fish, the main feature used to appreciate the freshness quality, is very important (Cheng *et al*., 2014). The hardness of chitosan coating+GTE showed lower changes than control samples due to antimicrobial properties of chitosan to inhibit the growth of bacteria (Shahidi *et al*., 1999; Kilinc *et al*., 2009;). Also, the bonds between chitosan and myofibrillar protein might be associated with the improvement of texture properties in fish muscle. The present findings are in agreement with Siripatrawan and Noipha (2012) who observed that application chitosan coating was effective to retard the change of texture parameters in pork

sausages during storage. Yuan *et al*. (2016) found that pretreatment of pacific white shrimp with chitosan as well as chitosan incorporating pomegranate peel extract could inhibit effectively increase in hardness. Li *et al*. (2013) reported that grape seed extract and tea polyphenol could extend the shelf life and improve the textural parameters of red drum. In the present study, the hardness and springiness of fish treated by chitosan coating+GTE was significantly improved. The springiness defines ability of the sample to recover its original form after removal of the deformation force. Springiness of fillet treated with Chitosan coating, GTE and chitosan+GTE with increasing storage was lower than that of the control.

The higher texture, odor, color, flavor and overall acceptance scores were found in fish coated with chitosan incorporation with GTE, followed by those coated with GTE and chitosan samples separately. Sample was considered as acceptable when the score was higher than 3. At day 16 of storage, the control samples had the overall likeness score of 2. Thus, this sample was considered as unacceptable or borderline for acceptability. For other samples coated with chitosan, GTE and chitosan+GTE, the overall likeness score was higher than 3. This was likely the result of the lowered spoilage of samples as indicated by the lower increase in bacterial load, chemical changes, color and texture changes (Siripatrawan and Noipha, 2012) compared with the control samples. The results suggested that samples coated with chitosan incorporation with GTE could increase the antioxidant and antimicrobial properties of the chitosan and thus maintained the qualities and prolonged the shelf life of the fish.

The results have been presented in this study indicated that chitosan coating combined with GTE could prohibit the bacteriological changes, chemicals and sensory qualities of the fish by retarding the lipids oxidation and microbial growth better than chitosan- alone coating and GTE- alone coating. The antioxidant and antimicrobial properties of the chitosan coating could be enhanced by incorporation with GTE. These combined preservation technologies have a broad application in the storage of tiger-tooth croaker.

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