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Effect of green tea (*Camellia sinenses*) extract and onion (*Allium cepa*) juice on lipid degradation and sensory acceptance of Persian sturgeon (*Acipenser persicus*) fillets

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Abstract: This study was established to contrast the effectiveness of red onion (*Allium cepa*) juice and green tea (*Camellia sinenses*) extract on lipid oxidation and sensory characteristics of refrigerated sturgeon fillets (*Acipenser persicus*). For this, fillets were tumbled in 1%, 2.5%, and 5% (v/v) aqueous solutions of onion Juice (OJ) and tea extract (TE), and then stored for up to 8 days at 4°C. Chemical indices of lipid oxidation as assayed by heme iron, thiobarbituric reactive substances (TBARs) and free fatty acid (FFA) contents indicated much more reduction in 2.5%TE, 5%TE and 5%OJ-treated samples relative to other samples ($P<0.05$). For 5%OJ treatment, the pH remained constant during storage ($P>0.05$) while gradual changes were detected in pH values of other treatments. Generally, the order of effectiveness for inhibiting the oxidation in fillets was found to be: 5%TE=5%OJ = 2.5%TE > 2.5%OJ>1%TE = 1%OJ. Based on sensory scores, higher amounts of onion juice (>1%) were more effective to improve attributive characteristics of fillets.

Keywords: Green tea, onion, sturgeon fillets, lipid degradation

Introduction

Lipid oxidation is a major cause of muscle food deterioration (Ladikose and Lougovoise, 1990), subsequent off-flavours, unpleasant odours, texture discolouration and nutritious value decrease (Frankel, 1998). It may be augmented by light, heat, metal ions and salt in fish flesh (Morrissey and Kerry, 2004). Addition of antioxidant with synthetic or natural origin is one of the strategies to reduce or retard oxidation and prevent the loss of quality and sensory attributes (Serdarglu and Felekoglu, 2005). Recently, the most commonly used synthetic antioxidants such as BHT, BHA and TBHQ (USDA, 2005) have been under attack due to their potential action in carcinogenesis (Imaida *et al.*, 1983) which led to the rejection of these imperfect additives by consumer preferences (Sherwin, 1990). Thereafter, there is a growing interest in the use of natural plants because of their considerable role as functional and biochemical inhibitors of oxidative damage induced by free radicals. Many plant tissues are good sources of phytochemicals, notably phenolic and flavonoids (Gorinstein *et al.*, 2005) that can act as the best alternatives to these mutagenic food additives. However, there might be wide differences in composition, concentrations and antioxidative properties in bioactive compounds of fruits and vegetables (Yang *et al.*, 2004).

Because of the current interest in health food, antioxidative properties of red onion (Allium cepa Linne.) as a ubiquitous aromatic plant and herbal green tea (*Camellia sinensis*) have been demonstrated in various studies (Helen *et al*., 2000; Kawamoto, 2004). Phenolic compounds in red onions including anthocyanins, flavonoids, quercetin and volatile sulfuric components show chelating and free radical scavenging activities (Zielinska *et al*., 2008), resulting in shelf life extension of fat-containing food systems. According to data published by Hertog *et al*. (1992), red onions exhibited the highest quercetin level (284-486 mg kg^{-1}) in a survey of 28 vegetables and 9 fruits. Also, Green tea leaves (*Camellia sinensis* L.) contain other strong well known antioxidant components, in that, catechins have been shown to minimize the oxidize ability of fatty acids by chelating iron and copper which cause the disruption of metal-catalyzed free radical formation (Chander *et al.*, 2005). It has been detected that trolox and other natural antioxidant compounds such as caffeic acid, cherogenic acid,

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quercetin, rutin and catechins are stronger scavengers as compared to vitamins C and E (Qu *et al.*, 2001). Remarkably, an increasing volume of research during the past decade has provided the evidence that flavonoids in these two widely consumed food plants exert many beneficial properties on human health like hypochlostrolomic, hypolemidemic, hypoglycaemic, thrombolitic, potent anticancer and cardiovascular effects (Yang *et al*., 2001; Higdon and Frei, 2003; Campos *et al.*, 2003).

Persian sturgeon (*Acipenser persicus*) is a commercially best-sold fish in modern retail outlets and the presence of high concentrations of labile phospholipids in fillets makes it more susceptible to oxidative deterioration. To date, few reports on sensory and chemical changes of sturgeon fillets have been available to determine the acceptable time for storage under different conditions. The overall objective of the present study was to elucidate the feasibility of red onion (*Allium cepa* Linne) and green tea (*Camellia sinensis*) on lipid peroxidation and quality control of sturgeon fillets during refrigerated storage with the preservation and enhancement of sensory properties. The other specific objectives of this work were: (a) to compare the effectiveness of these two plants with naturally occurring antioxidative components, and (b) to optimize the level of green tea extract and onion juice addition to the fish fillet.

Material and Methods

Chemicals and reagents

Sodium sulphate, aceton, tba-i reagent, phenolphthalein, acetic acid, sodium carbonate, tannic acid, folin-Ciocalteu´s phenol reagent and hydrochloric acid were prepared from Merck (KGaA, 64271 Darmastadt, Germany).Sodium hydroxide, ethanol and chloroform were purchased from Sigma (St. Louis, MO, USA).

Plant sample

Fresh red onions (*Allium cepa L.*) were purchased from a local grocery and the flesh part was used in the trail after peeling. Fresh leaves of *Camelia sinensis* were obtained from a retail market. To prepare ground dry green tea, the leaves were steamed (at 90±5°C) for 30 seconds, immediately cooled in iced-bowls and then reduced in size to give 80-mesh size powder. The crushed leaves were dried at 60 for 2 hours, and re-powdered in a burr mill.

Extraction of tea extract

Ten gram of ground dry green tea was added to 100 ml of distilled water and heated at 30-40ºC for 45 min with a magnetic stirrer (DELTA Model HM-101, Industries LTD).The mixture was then filtrated with a Wattman filtration paper No.42 and the filtered solution with soluble solid content was applied as green tea extract (TE) in the experiment.

Extraction of onion juice

Fifty gram of finely chopped red onions (*Alium cepa* L.) were thoroughly agitated with 500 ml of preheated water (90ºC) for 60 min in order to extract the highest content of phenolic compounds. The mixture cooled to room temperature and was homogenized in a blender (Pars Khazar, 320, Iran). The homogenate centrifuged at 10,000 rpm for 20 min in a high speed centrifuge (Sigma 3K30, Germany) and the resulting supernatant was used as onion juice (OJ).

Determination of total phenolics

Total phenolics were determined by a colorimetrical method of Folin-Ciocalteu reagent (Singh *et al*., 2002). The green tea extract and onion juice at each prepared concentrations were dissolved in 80% aqueous methanol (2: 1 v/v). 0.5 ml of the solution was well mixed with 1ml of diluted Folin-Ciolcalteu reagent (1:10 with distilled water) and 0.8 ml of 7.5% Na_2Co_3. The mixture was allowed to stand for 30 min at room temperature and their absorbance was measured at 765 nm with a spectrophotometer (Lightwave S2000 UV/VIS diode array spectrophotometer). The standard curve was prepared using 10,20,30,40,50,60,70, and 80 mgL^{-1} solutions of tannic acid in methanol: water (80:20, v/v). Total phenol values were expressed in terms of tannic acid equivalent (TAE) (mg g^{-1} of dry mass for green tea and fresh weight for red onion). The estimation of phenolic content was replicated three times and the results were averaged.

Sample preparation

Fresh sturgeon fish were caught from the northeast coast of Iran and transported to the laboratory in boxes containing enough ice within 2 hours *post mortem*. The average weight and total length of the fish were 12±2 kg and 80±5 cm. They were headed, eviscerated, skinned and filleted by using common household methods in medium length of 15±1 (cm) and weight of 350±5 (g). Preparation of the antioxidant solutions was performed freshly prior to fish catching in the laboratory. Different aqueous solutions of each plant extract were provided by dilution in distilled water at concentrations of 1%, 2.5%, and 5% (v/v). Fillets were tumbled in prepared dipping solution in a ratio 1:1 (w/v) for 10 min. After packaging with polyvinylidene film, the samples were placed in

Styrofoam trays and stored at 4 ºC for up to 8 days. The analysis of lipid oxidation indices and sensory characteristics was conducted at 5 intervals (0, 2, 4, 6 and 8 days).

Chemical analysis

The pH value was recorded using a pH meter (Metrohm, 713ph Meter-Herisau Switzerland), according to the method of Benjakul *et al*. (1997). Fish sample (0.5g) was centrifuged thoroughly with 4.5 ml of distilled water for 1 min at 1800 rpm and the homogenate used for pH determination. The lipid was extracted by the method of Bligh and Dyer (1959). Peroxide value (PV) was calculated according to the method of Egan *et al*. (1997) and the results were expressed as meq oxygen kg^{-1} lipids. Fish sample (150 g) was chopped well and agitated thoroughly with chloroform (250 ml) to dissolve the fat. The sample was filtered through Whatman filter paper No.42 containing sodium sulfate. Acetic acid (37 ml) and saturated potassium iodide solution (1 ml) was added to 25 ml of the filtrate. In order to obtain the weight of oil sample, 15 ml of the filtrate was incubated at room temperature to allow the chloroform removed, heated under an electric oven (105°C) for 1 hour, cooled in a desiccator, and then weighted. An adequate amount of 1% starch glue was mixed with the solution. After addition of distilled water (30 ml), the solution was transferred into the burette of an automatic titrator (DL 25 Titrator, Switzerland) equipped with stirrer. The titration was allowed to run against standard solution of sodium thiosulfate to give a milky color. PV was calculated as following:

$$PV=\frac{S\times N\times 1000}{W}$$

Where S is the volume of titration (ml), N the normality of sodium thiosulfate solution (N = 0.01), and W the weight of oil sample (g).

The thiobarbituric acid reactive substances (TBARs) content was determined using the method of Tarladgis (1969) and expressed as mg malonaldehyde kg^{-1} of fish flesh.10 g of comminuted fish flesh was homogenized with of distilled water (100 ml), HCL (4M, 2.5 ml) and 6-7 droplets of antifoaming. The mixture was subjected to the distillation process for 10 min. The obtained liquid (5 ml) was added to 4 ml of a TBA solution (0.0288 g TBA agent and %90 acetic acid), heated in a boiling water bath for 30 min, and then cooled. The concentrations of TBARs in samples were calculated by measuring the color development at 538 nm as following, where D is the absorbance of the solution against the blank sample preparing by adding 5 ml of distilled water and 5 ml of TBA solution.

TBARS (mg malonaldehyde kg^{-1}) =7.8×D

Free fatty acid (FFA) content was measured as described by Egan *et al*. (1997). Results were expressed as % oleic acid (i.e. the cm^3 0.1 N NaOH used in the titration corresponds to % oleic acid). Determination of heme iron content was conducted as described by Clark *et al.* (1997). 2 g of fish sample was added to 9 ml of acetone acid containing 90% acetone, 8% distilled water and 2% HCl with the normality of 12,7. After keeping in a small dark chamber for 30 min, the heme iron content was calculated using the following equations where A is the absorbance of the sample against a blank of acetone acid at 640nm.

[1] Total pigment (ppm) = A×680

[2] Heme iron (ppm) = total pigment×8.82/100

Sensory analysis

Sensory analysis (taste, colour and odour) were assessed according to the descriptive sensory method of Hedonic (ASTM, 1969) with slight modifications. A panel of 10 assessors was trained to evaluate each attribute and score them on a falling scale consisting of five points according to the guidelines in Table 1 at each sampling occasion. Fillets of fish were cooked in hot water steam for 20 min and the panelists were asked to wash their mouths with fresh water for several times before taste scoring.

Statistical analysis

The experimental design was a factorial 7×5×3 (7 treatments including the control, 5 sampling occasion, and three replicates). The chemical and sensory data were subjected to one way ANOVA when α=0.05. Comparisons within each analysis day and within a treatment at different sampling time were performed by least significant difference (LSD) test with statistical analysis system (SAS) program (α=0.05).

Results and Discussion

The mean compositional contents of moisture, protein, lipid and ash) in fresh fillets of sturgeon were assessed by the method of AOAC, 1995. As shown in Figure 1. The moisture, protein, lipid and ash contents of fresh sturgeon fillets were estimated to be 65.88%, 1.19%, 21.52% and 12.74%, respectively.

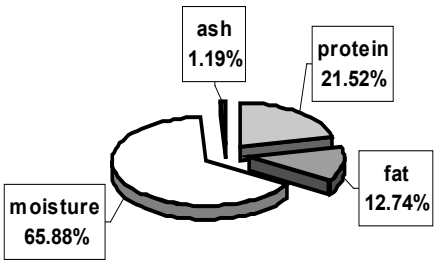

Figure1. Pie diagram showing the proximal composition of Persian sturgeon fillets

Phenolic content of plant extract

Total phenolic content of extracts at different concentration in terms of tannic acid equivalent (the standard curve equation: $y=0.00075x + 0.0091$, $r^2 = 0.9945$) is indicated in Table 2. Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992). In the present trial, the lowest (3.13 mg TAE g^{-1} dw) and highest (538.2 mg TAE g^{-1} dw) content were found in aqueous solutions of 1% OJ and 5% TE, respectively. According to the results in Table 2, green tea extract has much more content of phenolic compounds when compared to onion juice at the same concentration. Benkeblia (2000) reported the total phenolics of red onion var. Rouge Amposta from 18 to 20 mg $100g^{-1}$ fresh weight. Phenolic content in onions varies considerably particularly with cultivar (Bajaj *et al.,* 1980). As evidenced by Samman *et al*. (2001), the total phenolic compounds of green tea extracts were 117.3 mg gallic acid equivalents/g. It has been concluded that brewing conditiond such as extraction temperature, period of extraction, ratio of tea leaves to extracting water, and stirring are important factors for determining the total phenolic content in green tea extract (Liebert *et al*., 1999).

Changes in pH content

Changes in pH value of sturgeon fillets dipped in antioxidant solutions and the control are shown during 8 days storage in Table 3. The pH value assessed as a crucial factor for determination of meat quality (Nam *et al.,* 2001), might interfere with solubility activities of antioxidants by changing in their electrical charges (Decker *et al.,* 2005). In the present study, a gradual increase in the pH value of fish fillets was observed during 8 days of storage ($p<0.05$) and reached to the maximum of 7.19 at the end of sampling time (day8). The increase in pH was postulated to be due to an increase in volatile bases compounds produced by either endogenous or microbial enzymes (Cann *et al*., 1983), and decomposition of nitrogenous components (Benjakul *et al*., 2002). It has been emphasized that the pH value can be raised to pH 7 or 8 during storage period (Poulter and Nicolaides, 1985). Additionally, Sikorski *et al.* (1990) reported that the enzymatic degradation of ATP caused the liberation of inorganic phosphate and ammonia, leading to the changes in pH value. The most significant ($P<0.05$) differences among various treatments and within each analysis day were observed through the first 2 days of storage time whereas the pH content of treated fillets and the control exhibited no significant ($P>0.05$) difference on days 4, 6 and 8. As storage time increased, the pH values of 5% OJ-treated samples showed no change while gradually irregular increases were detected in other samples, suggesting a degree of stability in pH by an increase in the amount of green tea extract added (Table 1).

Changes in PV content

The effects of green tea extract and onion juice dipping solutions on the changes in the PV content of the sturgeon fillets during 8 days storage at 4°C are shown in Table 4. Peroxide value (PV) which detects a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation is widely used for the estimation of oxidative rancidity in fats (Ólafsdóttir *et al*., 1997). The PV values rose sharply during storage time in all samples and significant differences was recorded within all treatments and the control at each sampling time. A slower increase in PV values was obtained in samples treated with onion juice and green tea extract, in contrast to a faster increase in PV of the control sample after 4 days of storage time, demonstrating the oxidative stability of fish lipids by green tea extract and onion juice. The PV values of samples dipped in 5% OJ (4.75 meq oxygen kg^{-1} lipids) and 5% TE (4.28 meq oxygen kg^{-1} lipids) were approximately 1.7 and 1.8 times less than that of samples treated with 1% OJ (8.06 meq oxygen kg^{-1} lipids) and 1% TE (7.81 meq oxygen kg^{-1} lipids), respectively, at the end of storage time, which indicated that a high concentration of green tea and onion juice was more effective in controlling oxidation. Pervious results by Alghazeer *et al*. (2008) confirmed that addition of instant green tea (250 and 500 ppm) to Atlantic mackerel (*Scomber scombrus*) fillets decreased the rate of peroxides and hydroperoxides formation during 8 weeks storage at -10°C, as compared to samples without green tea. However, it has been reported that a higher concentration of green tea may act as a pro-oxidant (Honglian and Etsuo, 2001).

Table1. Descriptive sensory evaluation definitions

Sensory attributes description	score
natural flavor, color and odor of fillet	5
No sensible change in natural flavor, color and odor	4
Sensible discoloration, Slightly sour odor and incipient rancidity in flavour	3
no natural color, moderately off-odor and off-flavor	2
Sharply sour and extremely rancid flavor/odor, extremely discolored	1

Sourced by ASTM, 1969

Table 2. Phenolic content of green tea extract (TE) and onion juice (OJ) at different concentration

	Phenolic content(mg TAE/gdw)*		
TE 1%	145.6±0.037	OJ 1%	15.40±0.031
TE 2.5%	243.6±0.012	OJ 2.5%	37.39±0.022
TE 5%	538.2±0.055	OJ 5%	85.76±0.04

*mg Tannic acid equivalents per gramme dry weight of sample

Table 3. Changes in pH value of sturgeon fillets tumbled in tea extract (TE) and onion juice (OJ).

	Storage time (days)				
	0	2	4	6	8
1%TE	$6.62(1.40)_{c}^{BCD}$	$6.63(1.41)_{bc}^{CD}$	$6.54(1.38)_{c}^{AB}$	$6.85(1.46)_{a}^{A}$	$6.84(1.46)_{ab}^{A}$
2.5%TE	$6.51(1.38)_{c}^{D}$	$6.60(1.40)_{bc}^{D}$	$6.58(1.40)_{c}^{AB}$	$6.84(1.46)_{a}^{A}$	$6.81(1.45)_{ab}^{A}$
5%TE	$6.72(1.43)_{b}^{ABC}$	$6.74(1.43)_{b}^{ABC}$	$6.55(1.39)_{b}^{AB}$	$6.83(1.46)_{ab}^{A}$	$7.19(1.56)_{a}^{A}$
1%OJ	$6.59(1.40)_{b}^{CD}$	$6.73(1.43)_{ab}^{ABCD}$	$6.60(1.40)_{b}^{A}$	$6.81(1.45)_{a}^{A}$	$6.92(1.48)_{a}^{A}$
2.5%OJ	$6.84(1.46)_{ab}^{A}$	$6.79(1.45)_{ab}^{AB}$	$6.60(1.40)_{b}^{A}$	$6.50(1.38)_{b}^{B}$	$7.11(1.53)_{a}^{A}$
5%OJ	$6.80(1.45)_{a}^{AB}$	$6.83(1.46)_{a}^{A}$	$6.60(1.40)_{a}^{A}$	$6.82(1.46)_{a}^{A}$	$7.01(1.55)_{a}^{A}$

*Results are expressed as means with standard error in parenthesis (n=3).Different superscript and subscripts letters characterize significant difference in each column (A-D) and each raw (a-e), respectively.

Table 4: Comparison of Peroxide value in sturgeon fillets tumbled in green tea extract (TE) and onion juice (OJ).

	Storage time (days)				
	0	2	4	6	8
1%TE	$2.40\pm0.10_{e}^{B}$	$3.26\pm0.10_{d}^{B}$	$4.68\pm0.24_{c}^{B}$	$6.28\pm0.09_{b}^{B}$	$7.81\pm.0.14_{a}^{B}$
2.5%TE	$2.32\pm0.07_{d}^{BC}$	$2.50\pm0.20_{d}^{C}$	$3.26\pm0.12_{c}^{D}$	$4.48\pm0.09_{b}^{D}$	$4.81\pm0.21_{a}^{D}$
5%TE	$2.21\pm0.09_{e}^{C}$	$2.51\pm0.12_{d}^{C}$	$3.06\pm0.11_{c}^{D}$	$3.64\pm0.14_{b}^{E}$	$4.28\pm0.11_{a}^{E}$
1%OJ	$2.71\pm0.09_{e}^{A}$	$3.89\pm0.10_{d}^{A}$	$4.84\pm0.21_{c}^{B}$	$6.42\pm0.18_{b}^{B}$	$8.06\pm0.27_{a}^{B}$
2.5%OJ	$2.70\pm0.10_{e}^{A}$	$3.34\pm0.27_{d}^{B}$	$3.96\pm0.12_{c}^{C}$	$4.83\pm0.07_{b}^{C}$	$6.12\pm0.08_{a}^{C}$
5%OJ	$2.30\pm0.09_{e}^{BC}$	$2.75\pm0.05_{d}^{C}$	$3.32\pm0.08_{c}^{D}$	$3.80\pm0.14_{b}^{E}$	$4.75\pm0.21_{a}^{D}$

*Results are expressed as means with standard error in parenthesis (n=3).Different superscript and subscripts letters characterize significant difference in each column (A-D) and each raw (a-e), respectively.

Changes in TBA content

Table 5 depicts the formation of malonaldehydes in fillets of sturgeon during 8 days of refrigerated storage. The level of tissue aldehydes, the secondary degradation products of lipid oxidation as a result of peroxide breakdown into smaller molecules, is often assessed in biological systems (Khayat and Schwall, 1983). As shown in table 3, the formation of malonaldehydes was significantly retarded in samples treated with plant extracts. Up to sixth day of storage, significant ($P<0.05$) differences were observed in the TBARs content of the treatments at all sampling time except for samples dipped in 5% TE. There were no significant ($P<0.05$) differences among treatments of 2.5% OJ, 5% OJ, 2.5% TE and 5% TE at all analysis times while a significant difference ($P>0.05$) verified between samples treated by 2.5% OJ and 5% TE at the end of storage time. As shown in Table 5, samples treated by 1% OJ indicated no difference ($P>0.05$) in contrast to the control for all sampling time except day 4. Conversely, according to the results demonstrated by Serdaroglu and Felekoglu (2005), adding of onion juice (1ml: 100 g) to frozen sardine mince (*Sardina pilchardus*) delayed lipid oxidation for 3 months, although the TBARs values in both OJ and control treatments were out of consumable limits at the end of 5 month storage. As deduced by the comparison of thiobarbituric reactive substances content, antioxiadant efficiency of onion juice and tea extract with the same concentration were similar ($P>0.05$) in sturgeon fillets on each sampling occasion. Sturgeon fish which used in this study is a highly-fat fish with nearly 13% lipid content and prone to lipid oxidation. At the 8^{th} day of storage time, the lowest amount of malonaldehyde was assessed in refrigerated fillets tumbled in 5% TE. The minimum TBARs value for fillets dipped in higher concentrations of green tea extract well suggest the positive correlation between phenolic content of green tea extract and antioxidant properties of these compounds to prevent or retard the formation of malonaldehydes. In an investigation by Tang *et al*. (2001), tea catechins added at concentration greater than 300 mg kg^{-1} were necessary to reduce lipid oxidation in mackerel patties as indicated by significant decrease ($P<0.05$) in TBARs content. However, previous report by Van Het Hof (1997) revealed that green tea extract applications have no prohibition influence on lipid deterioration by using TBARs and MDA analysis.

Changes in FFA content

Variations in free fatty acid content of sturgeon fillets are depicted in Table 6. Progressive oxidation and enzymatic hydrolysis of unsaturated fatty acids is the main cause of lipid deterioration in fatty fish which is accompanied by the formation of free fatty acids (Srikar and Hiremath, 1972). In the present trail, lipid hydrolysis as measured by FFA indicated a general increase for all treatments including the control during refrigerated storage. This may be due to the effect of lipid hydrolyzing enzymes (mainly lipase and phospholipase) in decomposing the fats of fish tissue (phospholipids and triglycerides) during the first stages of refrigerated storage (Serdaroglu and Felekoglu, 2005). No significant ($P>0.05$) differences in the FFA content of various treatments were observed during the first 2 days of storage. For the latter refrigerated storage time, significant ($P<0.05$) differences were considerably detected among different samples treated by plant extracts; therefore different inhibitory effects of phenolic compounds on lipid hydrolysis of refrigerated sturgeon fillets could be implicated, depending on the amount of plant extract added. As shown by marked increase in free fatty acids, significant degradation of n-3 PUFA was observed in control samples after 8 days of refrigerated storage (Table 6). By the end of storage time, FFA content of the samples including 2.5% TE, 5% TE and 5% OJ were 2.13%, 2.75% and 2.31% lower than that of control, respectively. These data demonstrates that tumbling of fish fillets in green tea extract and onion juice at concentrations mentioned above, were more effective for preventing free fatty acids formation as compared with the others. Kumudavally *et al*. (2008) perceived that the application of sprayed-green tea extract (10ml kg^{-1}) could extend the shelf life of fresh mutton for up to 4 days at 25°C when registered nearly a 25% increase in FFA content of samples treated with green tea extract at day 4 against a 83% increase in the control sample at the end of 1 day storage. Serdarglu and Felekoglu (2005) noted that rosemary extract (100 ppm) showed better inhibition of lipid degradation than onion juice (1ml $100g^{-1}$) in Sardine (*Sardina pilchardus*) mince as assayed by more increases in FFA content of samples with 1%OJ (v/w).

Changes in Heme iron content

The changes in heme iron content of sturgeon fillets during 8 days are presented in Table 7. Fish flesh is a source of iron-containing complexes including, myoglobin, haemoglobin and iron-bound proteins such as ferritin and transferin (Hazell, 1982). Processing techniques such as filleting and mincing as well as during storage time can led to the release of this iron from the complexes, and then catalyze lipid autoxidation in fish muscle (St. Angelo, 1996). As indicated in , heme iron content increased in the

Table 5. Comparison of thiobarbituric reactive substances (TBARs) content in sturgeon fillets tumbled in green tea extract (TE) and onion juice (OJ).

	Storage time (days)				
	0	2	4	6	8
1%TE	$0.14\pm0.01^{A}_{d}$	$0.27\pm0.08^{AB}_{d}$	$0.60\pm0.06^{BC}_{c}$	$1.16\pm0.17^{BC}_{b}$	$1.84\pm0.09^{ABC}_{a}$
2.5%TE	$0.13\pm0.01^{A}_{c}$	$0.24\pm0.03^{B}_{c}$	$0.30\pm0.09^{D}_{c}$	$0.61\pm0.13^{D}_{b}$	$0.97\pm0.09^{DE}_{a}$
5%TE	$0.11\pm0.02^{A}_{b}$	$0.20\pm0.00^{B}_{b}$	$0.28\pm0.07^{D}_{b}$	$0.51\pm0.12^{D}_{ab}$	$0.80\pm0.25^{E}_{a}$
1%OJ	$0.14\pm0.01^{A}_{d}$	$0.31\pm0.01^{AB}_{cd}$	$0.70\pm0.06^{B}_{c}$	$1.34\pm0.27^{AB}_{b}$	$2.05\pm0.19^{AB}_{a}$
2.5%OJ	$0.12\pm0.01^{A}_{c}$	$0.29\pm0.03^{AB}_{c}$	$0.48\pm0.06^{BCD}_{bc}$	$0.79\pm0.14^{BCD}_{b}$	$1.50\pm0.29^{BCD}_{a}$
5%OJ	$0.12\pm0.00^{A}_{c}$	$0.25\pm0.02^{AB}_{bc}$	$0.37\pm0.11^{CD}_{bc}$	$0.70\pm0.11^{CD}_{b}$	$1.29\pm0.30^{CDE}_{a}$

*Results are expressed as means with standard error in parenthesis (n=3).Different superscript and subscripts letters characterize significant difference in each column (A-D) and each raw (a-e), respectively.

Table 6. Comparison of free fatty acids (FFA) in sturgeon fillets tumbled in green tea extract (TE) and onion juice (OJ)

	Storage time (days)				
	0	2	4	6	8
1%TE	$1.06\pm0.19^{A}_{d}$	$1.41\pm0.19^{B}_{d}$	$2.94\pm0.18^{AB}_{c}$	$3.98\pm0.16^{BC}_{b}$	$4.78\pm0.12^{B}_{a}$
2.5%TE	$1.10\pm0.10^{A}_{d}$	$1.29\pm0.44^{B}_{d}$	$2.23\pm0.22^{BC}_{c}$	$3\pm0.13^{D}_{b}$	$3.80\pm0.08^{CD}_{a}$
5%TE	$1.11\pm0.06^{A}_{c}$	$1.21\pm0.33^{B}_{c}$	$1.86\pm0.07^{C}_{bc}$	$2.85\pm0.54^{D}_{ab}$	$3.18\pm0.31^{D}_{a}$
1%OJ	$1.19\pm0.12^{A}_{c}$	$1.68\pm0.37^{AB}_{c}$	$2.97\pm0.15^{AB}_{b}$	$4.22\pm0.42^{AB}_{a}$	$4.77\pm0.29^{B}_{a}$
2.5%OJ	$1.23\pm0.13^{A}_{c}$	$1.73\pm0.26^{AB}_{c}$	$2.64\pm0.44^{B}_{b}$	$3.20\pm0.31^{DC}_{ab}$	$4.03\pm0.06^{C}_{a}$
5%OJ	$1.10\pm0.22^{A}_{c}$	$1.58\pm0.29^{AB}_{c}$	$2.59\pm0.26^{BC}_{b}$	$3.01\pm0.12^{D}_{ab}$	$3.62\pm0.41^{DC}_{a}$

*Results are expressed as means with standard error in parenthesis (n=3).Different superscript and subscripts letters characterize significant difference in each column (A-D) and each raw (a-e), respectively.

control and samples treated by 2.5% OJ, 2.5%TE and 5% TE after storage for 2 days. Afterwards, a significant ($P<0.05$) decrease were recorded which was especially sharp at day 6 for all treatments including the control. This could be as a result of the release of iron from heme to change into non-heme iron. In addition, measurement of heme iron content detected a significant ($P<0.05$) increase in the control and 2.5% OJ, 2.5% TE-treated samples during the last 2 days of storage time (days 6 and 8). Markedly, treatments with 2.5% TE, 5% OJ, and 5% TE had the highest content of heme iron content at the end of storage time which was 3.31, 3.34 and 3.12, respectively. This can be implicated to the positive effect of phenolic compounds presenting in considerable content in green tea extract added at concentrations more than 1% (v/v) while even onion juice with a low content of phenolic component showed the inhibition of iron release in sturgeon fillets. Upon extended refrigerated storage, a slower rate of decrease in heme iron content of fillets was found with increasing in the concentration of green tea extract. However, fillets tumbled in 5% TE showed the highest amount of heme iron content on most days of storage time as compared to other samples. Many in vitro studies have shown that metal-chelating properties of flavonoids in green tea have protective effects in cells and tissues against damages caused by free oxygen radicals (Kashima, 1999; Rietveld and Wiseman, 2003).

Changes in sensory attributes

Results of sensory difference testing in treated sturgeon fillets during 8 days storage at 4°C are shown in Figure 2. All treatments developed off-odour with increased storage time, with the lowest and the highest off-odour detected on samples treated with 5% TE and 1% OJ, respectively. Meanwhile, the early signs of off-odour appeared in 1% OJ-treated fillets and the control on day 4 but developed in other treatments after 6 days of storage time. Younathan *et al.* (2006) revealed that hot water extracts of yellow onion peels controlled rancid odour of cooked ground turkey even though TBARs numbers were high. Sensory properties

Table 7. Comparison of heme iron content in sturgeon fillets tumbled in green tea extract (TE) and onion juice (OJ).

	Storage time (days)				
	0	2	4	6	8
1%TE	$5.15\pm0.361_{ab}^{BC}$	$5.5\pm0.098_{a}^{C}$	$4.91\pm0.006_{b}^{CB}$	$2.56\pm0.058_{c}^{CD}$	$3.02\pm0.157_{c}^{BC}$
2.5%TE	$5.66\pm0.04_{b}^{A}$	$6.03\pm0.053_{a}^{A}$	$5.26\pm0.032_{c}^{B}$	$3.03\pm0.07_{e}^{B}$	$3.31\pm0.095_{d}^{AB}$
5%TE	$5.52\pm0.174_{b}^{AB}$	$6.21\pm0.058_{a}^{A}$	$5.53\pm0.07_{b}^{A}$	$3.5\pm0.058_{c}^{A}$	$3.26\pm0.083_{c}^{AB}$
1%OJ	$5.01\pm0.062_{a}^{C}$	$5.26\pm0.078_{a}^{CD}$	$4.46\pm0.235_{b}^{C}$	$2.69\pm0.167_{c}^{CB}$	$2.86\pm0.058_{c}^{CD}$
2.5%OJ	$4.94\pm0.117_{b}^{C}$	$5.44\pm0.062_{a}^{C}$	$4.93\pm0.162_{b}^{CB}$	$2.31\pm0.058_{d}^{D}$	$2.69\pm0.121_{c}^{D}$
5%OJ	$5.11\pm0.115_{a}^{BC}$	$5.1\pm0.115_{a}^{D}$	$5.1\pm0.362_{a}^{B}$	$2.98\pm0.147_{b}^{B}$	$3.34\pm0.058_{b}^{A}$

*Results are expressed as means with standard error (n=3).Different superscript ad subscripts letters characterize significant difference in each column (A-D) and each raw (a-e), respective.

of food products are the key factors in consumer attraction (Gray *et al*., 1996) and the implication of lipid peroxidation in flavour and aroma deterioration, as well as diminished food wholesomeness and food safety has been confirmed (Kanner and Rosenthal, 1992) Up to 4 days of storage time, no significant ($P>0.05$) differences were observed among treated samples with the discovery of off-flavour in the control (Figure 2). Most differences among various treatments including the control were recognized at 6th day when a sharp increase of off-flavour was detectable in 1% OJ and control samples. By the end of storage time, samples tumbled in 1% OJ, 1% TE and the control had the lowest flavour scores while treatments with upper concentrations of plant extracts were scored highly in flavour analysis. Tang and Cronin (2007) noted that the incorporation of onion juice during the preparation of encased turkey breast rolls led to the production of premium quality product processing and improved flavour quality and stability in sliced form. As stated by Scideman *et al*. (1984) meat colour is considered one of the most important quality attributes in the acceptance or rejection of the product. Refrigerated fillets treated by 2.5% TE, 5% TE and 5% OJ showed a much superior colour quality after storage for 8 days at 4 °C (Figure 2). Although increase in the concentration of green tea extract had beneficial effects on chemical changes of surgeon fillets particularly at the end of storage time, panelists characterized the new slightly bitter and greeny flavor with greenish-yellowish colour for 5% and 2.5% TE treatments at all sampling times. The discoloration changes of fish fillets dipped in 2.5% and 5% TE steadily increased with time in storage. This might be implicated to the possibly penetration of chlorophyll pigments and their subsequent interference with other biochemicaly active compounds in fillets of sturgeon, which caused an undesirable change in meat colour. Persian sturgeon is a white-muscled fish with pale pink

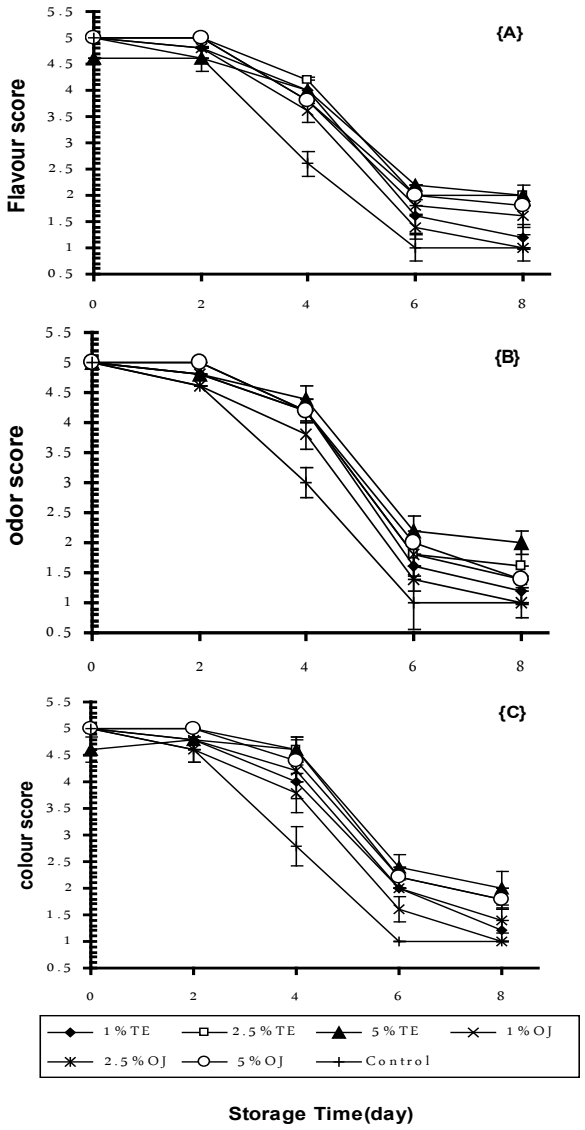

Figure 2. Comparative evolution of flavour (A), odor (B) and color (C) scores in sturgeon fillets treated by tea extract (TE) and onion juice (OJ) at three concentrations (1%, 2.5%, 5%) during refrigerated storage. Bars denote standard error of the mean (n=3).

flesh coloration after cooking by steam. This finding provides the evidence that tea extracts, particularly at upper concentrations, are good inhibitors to lipid damage progression but incapable of retaining and/or promoting the natural flavour and colour of sturgeon filltes. In this sense, Liu (1997) stated that clove had an antioxidative impact on lipid degradation of marinated catfish with a significant decrease in the sweetness taste of the meat.

Conclusion

We concluded that green tea extracts and onion juice in concentration upper than 1% (v/v) and 2.5% (v/v) respectively had more antioxidant characteristics on oxidative stability of lipids and shelf life enhancement in refrigerated sturgeon fillets which was more detectable with increased storage time. The present study dose not reflect a clear dose-relationship between the total phenolic content of plant extracts and the results obtained from the lipid oxidation indices assessed in refrigerated sturgeon fillets. However, colour and flavour of samples with 5% TE were unfavorable and might not be appealing to consumers. For further evaluation, comparison of green tea extract and onion juice from various varieties, by different extraction methods, and at other concentrations is proposed with the employment of microbiological analysis which has not been investigated in the present trail.

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