

## Influence of Plant Antioxidant on the Shelf-life of Seafood

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### Abstract

Fish is one of the principal protein sources in the human diet. From an economic standpoint, people want to meet their need for animal proteins from cheaper food. As a result of the increasing number of working women and rapid urbanization, the demand for ready-to-serve food products is rising. Ready-to-serve food products do not spoil for a period of time due to the processing techniques used. The natural antioxidant system is divided into two major groups, enzymatic and non-enzymatic antioxidants. Regarding enzymatic antioxidants they are divided into primary and secondary enzymatic defences while the primary defence is composed of three important enzymes that prevent the formation or neutralise free radicals: glutathione peroxidase, catalase, superoxide dismutase and the secondary enzymatic defence includes glutathione reductase and glucose-6-phosphate dehydrogenase.

**Keywords:** Ascorbic acid, antimicrobial, rosemary, extracts, antioxidants

### Introduction

Fish is one of the principal protein sources in the human diet. From an economic standpoint, people want to meet their need for animal proteins from cheaper food. As a result of the increasing number of working women and rapid urbanisation, the demand for ready-to-serve food products is rising. Ready-to-serve food products do not spoil for a period of time due to the processing techniques used. These products are consumed either directly or after being heated, and may be served either alone or with other products (Gokoglu 1994) [37].

### Plant natural antimicrobial compounds

Essential oils and plant extracts obtained from aromatic medicinal plants have been reported to show singularly good antimicrobial effects against bacteria, filamentous fungi, yeasts, and viruses. These are very complex natural mixtures including hydrocarbons (mainly terpenoids) and oxygenated compounds (alcohols, ethers, esters, ketones, aldehydes, lactones, phenols and phenol ethers (Stefanakis *et al.* 2013) [38]. The compositions of EOs and plant extracts from a particular species of plant can differ between geographical sources and harvesting seasons. In general, EOs produced from herbs harvested during or immediately after flowering possesses the strongest antimicrobial activity (Burt 2004) [9]. These compositions can constitute a powerful tool to reduce the development and dissemination of antimicrobial resistance. The means by which microorganisms are inhibited by phenolic compounds involves a sensitization of the phospholipid bilayer of the cell membrane, causing an increase in permeability and leakage of vital intracellular constituents, or impairment of bacterial enzyme systems. Phenolic compounds act by inhibiting the amino acid decarboxylase in target bacteria (Ojagh *et al.* 2010) [27]. Some studies expressed that plant extracts and EO components appear to make the cell membrane permeable and are able to disintegrate the outer membrane of Gram-negative bacteria and these are slightly more active against Gram-positive than Gram-negative bacteria (Burt 2004; Abdollahzadeh *et al.*

2014) [9, 39]. However, not all researches on EOs have concluded that Gram-positive bacteria are more susceptible (Wilkinson *et al.* 2003) [36]. The antimicrobial activity of essential oils and plant extracts would be related to the respective composition and structural configuration of the plant volatile oils, their functional groups and possible synergistic actions and reactions between components (Mahmoodi *et al.* 2012) [24]. Phenolic compounds comprise the main antimicrobial components in spices and their derived essential oils and extracts, and include, for instance, cinnamic aldehyde from cinnamon; thymol from thyme and oregano; eugenol from clove, allspice and cinnamon; carvacrol from oregano and anethole from anise (Mahmoud *et al.* 2004) [40]. As a result, natural antimicrobials are receiving a good deal of attention for a number of microorganism-control issues (Mahmoodi *et al.* 2012) [24].

### Rosemary (*Rosmarinus officinalis*)

Rosemary (*Rosmarinus officinalis*) is a small ever-green bush, belonging to the Labiatae family. It grows principally in the basin of the Mediterranean Sea, while in Poland it is usually cultivated in pots. Active substances present in *Rosmarinus* yield it a series of properties, desirable from the point of view of the food industry and medicinal phytochemistry (Rumińska and Ożarowski 1990, Djeddi *et al.* 2007) [33, 15]. The majority of data found in literature (Madsen and Bertelsen 1995, Beltran *et al.* 2004, Fernandez-Lopez *et al.* 2005, Moreno *et al.* 2006, Georgantelis *et al.* 2007, Pietrzak and Myron 2008) [23, 6, 17, 25, 18, 31] refers to the anti-oxidative properties of *Rosmarinus officinalis*. A rosemary extract, in the form of an emulsion, powder or oil solution, may be used as a substitute for BHA (butylated hydroxyanisole), added to dehydrated chicken eggs, meat and fish, while rosemary extracts may be added to sausages, macaroni, peanut butter and oil.

### Rosemary essential oils and extracts

Essential oils and extracts, both the aqueous and oil ones, obtained from rosemary are characterised by a high

antimicrobial activity. Bacterial strains especially susceptible to the activity of essential rosemary oils include: *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Klebsiella pneumoniae* (Mangena and Muyima 1999, Celiktas *et al.* 2007, Djeddi *et al.* 2007) [41, 12, 15]. Rosemary extracts display a relatively poor inhibiting effect on Gram-negative bacteria but, at a level of 0.06-1%, they inhibit the growth of Gram-positive pathogens, such as: *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus*. Moulds of the *Penicillium* and *Botrytis* genus are developing much slower in the environment containing a rosemary extract, while carnosol and carnosic acid (components of rosemary extracts) inhibit the vital activities of drug-resistant bacteria of the *Staphylococcus aureus* strain. Especially susceptible to the activity of rosemary extracts are also bacteria of the *Lactobacillus* and *Brochothrix* genus (Del Campo *et al.* 2000, Moyosoluwa *et al.* 2004, Fernandez-Lopez *et al.* 2005) [14, 26, 17].

### Usage of antioxidants

Usage of antioxidants and vacuum packaging have the best influence on increasing shelf-life and delaying improper changes in sea food (Serdaroglu & Felekoglu, 2005) [42]. Antioxidants block the formation of free radicals, stabilize hydro peroxides and thus slow down oxidation and rancidity development. Recently, the demand for novel natural antioxidants has increased; this is because of possible adverse side effects of synthetic antioxidants and beneficial effects of natural antioxidants (Benjakul *et al.*, 2005; Sarkardei & Howel, 2008) [7, 34].

### Natural antioxidant system

The natural antioxidant system is divided into two major groups, enzymatic and non-enzymatic antioxidants. Regarding enzymatic antioxidants they are divided into primary and secondary enzymatic defences while the primary defence is composed of three important enzymes that prevent the formation or neutralise free radicals: glutathione peroxidase, catalase, superoxide dismutase and the secondary enzymatic defence includes glutathione reductase and glucose-6-phosphate dehydrogenase. These two enzymes do not neutralise free radicals directly, but have supporting roles to the other endogenous antioxidants (Carocho & Ferreira 2013) [11]. The non-enzymatic endogenous antioxidants include vitamins (C, E, and carotenoids), polyphenols (ellagic acid, gallic acid, and tannins), flavonoids (flavones, isoflavones, flavonols, anthocyanins, and catechins) (Gupa & Sharma 2006) [20]. Reports have revealed plants to be rich sources of natural antioxidant compounds. Phenolic compounds are commonly found in plants, and have been reported to have a wide range of biological activities including antioxidant properties that directly or indirectly contribute to the inhibition or suppression of oxidation processes (Shahidi 2008; Cox *et al.* 2010) [35, 13]. Thyme and oregano EOs have two major constituents: as percentage of the total content are the phenols carvacrol and thymol. Carvacrol and thymol comprise the main antioxidant components. In addition, the flavonoids of oregano EO are a group of compounds with antioxidant activity (Goulas & Kontominas 2007; Kykkidou *et al.* 2009) [19, 22]. Rosemary has been reported to contain certain compounds such as rosmanol, rosmaridiphenol, rosmariquinone, and carnosol, which may be up to four times

equal to butylated hydroxyanisole (BHA) and as effective as butylated hydroxytoluene (BHT) as antioxidants (Cadun *et al.* 2008; Rohlík *et al.* 2010, 2013) [10, 43]. The antioxidant properties of turmeric extract are mainly attributed to the curcumin and phenolic compounds as well as these properties appear to be related to the high contents of sulphur-containing compounds, flavone, and polyphenolic derivatives in the shallot extract (Pezeshk *et al.* 2011) [30]. Cinnamon has a good antioxidant potential. This plant is rich in cinnamaldehyde as well as  $\beta$ -caryophyllene, linalool, and other phenolic compounds (Ojagh *et al.* 2010) [27]. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties, which can play an important role in neutralising and adsorbing free radicals, triplet oxygen, quenching singlet and/or decomposing peroxides (Bajpai *et al.* 2009) [4]. Phenolic compounds are ubiquitous in plants, and when plant foods are consumed, these phytochemicals contribute to the intake of natural antioxidants in the human diets (Balasundram *et al.* 2006) [5].

### Ascorbic acid (AA) and citric acid (CA)

Ascorbic acid (AA) and citric acid (CA) and their salts are widely known for their role as chelators (Boyd *et al.*, 1993; Oktar *et al.*, 2001; Kim *et al.*, 2006) [8, 28, 21] in biological systems and synergists of other antioxidants. The positive effects of AA and CA on fish oil and emulsions (Osborn-Barnes & Akoh, 2003) [44], minced fish (Stodolnik *et al.*, 1992; Abdelaal, 2001) [45] and fish fillets (Badii & Howell, 2002; Aubourg *et al.*, 2004; Pourashouri *et al.*, 2009) [3, 29, 32] have been observed. Vacuum packaging is another way for delaying lipid oxidation (auto oxidation) because of limiting oxygen molecule. As reported by Anelich *et al.* (2001) [1], Fagan & Gormley (2004) [16] and Perez-Alonso *et al.* (2004) [29], packaging under vacuum has positive effect on extended shelf life of fish fillets. In the present study, Persian sturgeon (*Acipenser persicus*) fillets were used.

### Material and methods

This article is review and the aims of influence of Plant Antioxidant on the Shelf-life of Seafood. The experiment 1 was conducted by Rostamzad *et al.* (2010) [47]. Fresh Persian sturgeon (*Acipenser persicus*) was captured and kept on ice (1h) till delivery to the laboratory. Then, the fish were gutted, dressed and filleted manually weighing 400-450 g. Then fillets were divided into 4 groups. First group was left untreated and directly packaged traditionally in polyethylene bags (control samples; BC treatment). Samples of the second group were packaged under vacuum conditions in polyethylene bags (VP treatment). The third group was immersed in 0.50% aqueous solution of Ascorbic acid (AA treatment) and fourth group was immersed in 0.50% aqueous solution of Citric acid (CA treatment). Then, samples were immediately frozen at -40 °C for 24 h and then kept in -18 °C. Samplings were carried out from the fresh fish (initial material) and then during frozen storage (at 1st, 3rd and 6th months). For each treatment (BC, AA, CA and VP), three different fish batches (totally 48 batches of fillets) were considered and examined individually. Chemicals (solvents and reactants) employed through the study were reagent grade (Merck, Germany). To measure the pH, five grams of fish mince was homogenized for 1 minute with 45 ml of distilled water. pH value was measured using a standardized portable pH meter (Metrohm). Expressible

moisture content was determined by weight difference between the mussel (1-2g) of fish before and after being pressed under 0.5 and 1 kg load for 5 and 20 minutes. Sensory analyses were conducted by a taste panel consisting of five to seven panelists, according to the guidelines presented in Table 1. Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and poor quality (C). Sensory assessment of the fish fillet included the following parameters: flesh appearance, rancid odor and flesh consistency (Table.1). At each sampling, the different fish fillets were thawed and then analyzed in the same session. The fish fillets were served to the panel members in individual polyethylene bags in which they had been kept frozen and they were scored individually. Sensory analyses were carried out at 0, 1, 3 and 6 months after storage. Three replicates were used for each experiment. Data from the different quality measurements were subjected to the ANOVA one-way method. Comparisons of means after the ANOVA test were performed using a least-squares difference (LSD) method. The experiment 2 was conducted by Yerlikaya and Gokoglu (2010) [48]. Bonito (*Sarda sarda*) was purchased from the main fish market in Antalya, Turkey and transferred to the laboratory in polystyrene boxes with crushed ice. The mean weight and length of fish were  $181.4 \pm 18.91$  g and  $27.96 \pm 1.35$  cm, respectively. Antique green tea leaves (*Camelia sinensis*) harvested in 2004 and 2005 shooting periods were purchased from the local market in Antalya and dried at 40 °C for 12 h in an oven (Yerlikaya and Gokoglu., 2010) [48]. All samples were then ground into a fine powder with a mill. The powders dissolved in ethanol (1:20 w/v) and then extracted in a water bath with shaker at 40 °C for 4 h. The extracts were filtered and concentrated in a rotary evaporator to get crude extracts. Grapes were purchased from the market in Antalya and seeds were manually separated. The seeds were dried at 50°C until a constant weight and ground to powder and extracted in a soxhlet extractor with petroleum ether for 6 h. Pomegranate (*Punica granatum*) were also purchased from the market and peeled. Pomegranate peels were dried at 50 °C until a constant weight and ground to powder (Yerlikaya and Gokoglu., 2010) [48]. Defatted seed powder and pomegranate peel powder were dissolved in ethanol (1:20 w/v) and then extracted in a water bath with shaker at 40°C for 4 h. The extracts were filtered and concentrated in a rotary evaporator to get crude extracts. All green tea, grape seed and pomegranate peel extracts were then stored under nitrogen at -20 °C until use. Extract solution was prepared by dissolving 1.0 g plant extract in 100 ml distilled water. Fillets were separately dipped into three extract solutions and frozen in an air-blast freezer at -40°C. Another group fillets were dipped into water as control. Frozen bonito fillets were wrapped using stretch film (10 micron thickness) and aluminum foil (20 micron thickness), placed in carton boxes and stored at -18 °C (Yerlikaya and Gokoglu., 2010) [48]. Quality control analyses were performed during the storage on monthly intervals. Frozen fillets were thawed in a refrigerator (at 4 °C) for each sampling time. Total phenolic contents of the extracts were determined according to the Folin-Ciocalteu colorimetric method. Each extract of 0.1 ml was introduced to 5 ml Folin-Ciocalteu's reagent (0.2 N), 4 ml sodium carbonate (7.5 g L-1) and 0.9ml distilled water. The mixture was allowed to stand for 2 hours before absorbance measurement against blank at 765 Nm (Yerlikaya and Gokoglu. 2010) [48].

## Results and Discussion

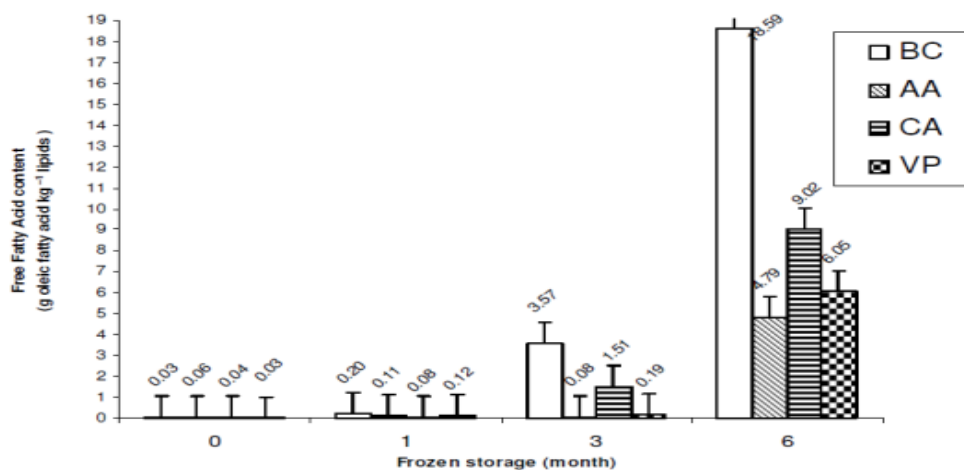
In the experiment 1 was conducted by Rostamzad *et al* (2010) [47]. Hydrolysis development (FFA content) increased ( $p < 0.05$ ) in all type of samples during frozen storage (Fig. 1). The antioxidant and vacuum packaging treatments led to lower value during the whole storage. Comparison of the different kinds of treatments led to higher ( $p < 0.05$ ) hydrolysis development at month 6 for BC samples while lower values were maintained throughout the whole experiment period for AA samples ( $p < 0.05$ ) (Rostamzad *et al.*, 2010) [47]. During frozen storage, a slow increase on the basis of primary oxidation products (peroxide values, PV) values was observed for each treatment, at sixth month a marked increase was observed for Blank control (Fig. 2) (Rostamzad *et al.*, 2010) [47]. In Blank control significant difference ( $p < 0.05$ ) were obtained at 3 and 6 months and antioxidants and vacuum packaging treatments only after 3 month had significant change ( $p < 0.05$ ) (Rostamzad *et al.*, 2010) [47]. From the results, it is concluded that all three treatments had significant effect on delaying lipid oxidation but AA and vacuum packaging were the most effective treatments among them. Secondary lipid oxidation products, as reported by the TBA-i, presented low values at the beginning of the study (Fig. 3). and gradually increased during frozen storage (as in the case of PV) (Rostamzad *et al.*, 2010) [47]. A significant increase in Thiobarbituric acid TBA-i value was observed for control and CA-treated samples ( $p < 0.05$ ) compared with the other treatments during storage. pH values ranged between 6.15 and 6.92 among samples and decreased at during storage at freezer but no statistical difference were observed among treatments and Blank control ( $p < 0.05$ ).

The initial pH value of treated samples was lower than that in their corresponding control samples and this lower value was maintained during the 3-6 months period. When vacuum packaging (VP) treatment samples showed a lower ( $p < 0.05$ ) pH value compared with other treatments, no significant differences were observed in the 3-6 months period among different treatments. Expressible moisture content showed a gradual increase for all samples during the course of the study (Rostamzad *et al.*, 2010) [47]. Comparison of the different treatments revealed that the antioxidant and vacuum packaging treatments had lower Expressible moisture content values compared to the Blank control but no significant differences were detected among the samples of antioxidant and vacuum packaging treatments throughout the whole experiment. Initially, odor, taste, color and appearance of fillet were natural and fresh. However, their quality deteriorated with time. Scores given to the four sensory indices decreased as storage time increased (Table 2). Flesh appearance assessment showed a lower ( $P < 0.05$ ) score at month 6 for the BC samples than other treatments. Odor analysis led to a better quality score ( $P < 0.05$ ) at month 3 for AA -treated samples than that for BC, CA and VP treatments. Flesh odor and flesh appearance in control samples at month 6 of storage was considered a limiting factor. Among different kinds of molecules produced as a result of lipid oxidation, secondary ones are considered the chief compounds responsible for oxidized flavors. A close relationship between the rancid odor development and the TBA-i assessment has been obtained in the present study. In the experiment 2 was conducted by Yerlikaya and Gokoglu (2010) [48].

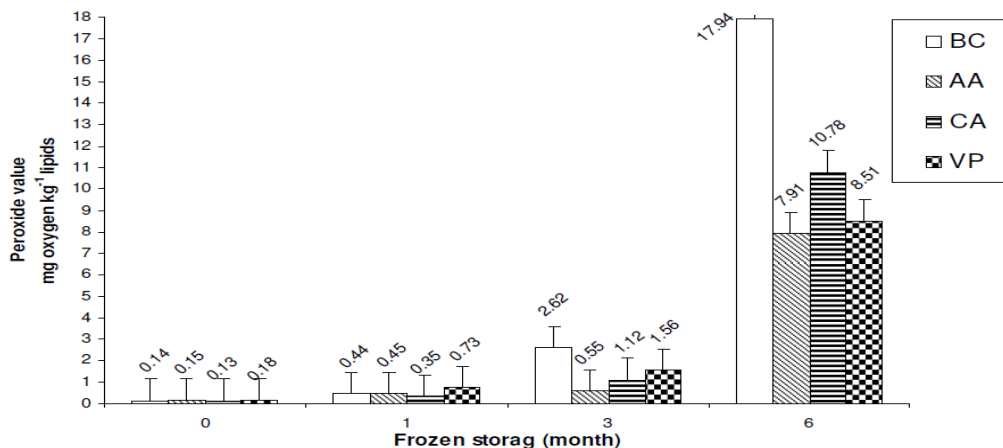
**Table 1.** Scale employed for evaluating the sensory quality of frozen Persian sturgeon fillets (Rostamzad *et al.*, 2010)<sup>[47]</sup>

Attribute	E (Highest quality)	A (Good quality)	B (Fair quality)	C (poor quality)
<b>Flesh appearance</b>	Strongly hydrated and pink; myotomes totally adhered	Still hydrated and pink; myotomes adhered	Slightly dry and pale; myotomes adhered in groups	Yellowish and dry; myotomes totally separated
<b>Rancid odor</b>	Sharp seaweed and shellfish	Weak seaweed and shellfish	Slightly sour and incipient rancidity	Sharply sour and rancid
<b>Flesh consistency</b>	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes as a result of mechanical factors

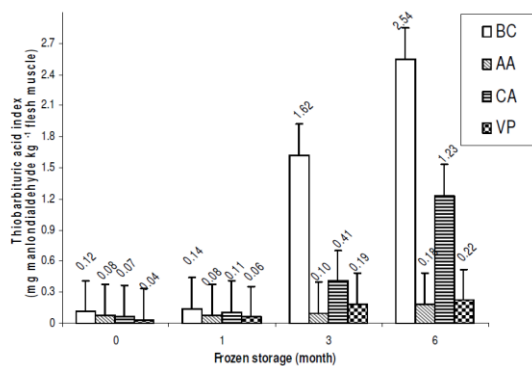
\*Adapted from DOCE (1989)



**Fig 1:** FFA content of the Persian sturgeon n fillet during frozen storage at -18 °C (Rostamzad *et al.*, 2010)<sup>[47]</sup>



**Fig 2:** PV content of the Persian sturgeon fillet during frozen storage at -18 °C (Rostamzad *et al.*, 2010)<sup>[47]</sup>



**Fig 3.** TBA content of the Persian sturgeon fillet during frozen storage at -18 °C (Rostamzad *et al.*, 2010)<sup>[47]</sup>

**Table 4:** Instrumental color scores of frozen bonito fillets previously treated with plant extracts (Yerlikaya and Gokoglu 2010) [48].

Colour Analysis		Storage Time (Months)				
Treatments		1	2	3	4	5
L Value	GT	48.3±1.2 <sup>aY</sup>	50.7±1.2 <sup>aXY</sup>	53.2±2.8 <sup>aX</sup>	50.7±1.1 <sup>aXY</sup>	49.2±0.7 <sup>aXY</sup>
	GS	47.0±0.8 <sup>aX</sup>	46.1±4.8 <sup>*</sup>	46.0±0.9 <sup>bXY</sup>	45.1±0.7 <sup>bXY</sup>	43.9±1.0 <sup>bY</sup>
	PP	51.8±2.2 <sup>aX</sup>	47.7±0.1 <sup>bXY</sup>	43.2±3.2 <sup>bYZ</sup>	41.1±1.5 <sup>cZ</sup>	40.9±1.4 <sup>cZ</sup>
	C	49.6±6.7 <sup>aX</sup>	47.0±0.3 <sup>bX</sup>	46.8±2.5 <sup>abX</sup>	47.2±0.8 <sup>bX</sup>	46.1±0.5 <sup>bX</sup>
a Value	GT	4.7±0.0 <sup>bX</sup>	5.9±0.4 <sup>bX</sup>	6.8±0.3 <sup>abX</sup>	5.7±1.1 <sup>abX</sup>	6.0±1.7 <sup>aX</sup>
	GS	9.1±0.0 <sup>aX</sup>	9.4±2.1 <sup>aX</sup>	8.5±0.1 <sup>aX</sup>	8.4±0.5 <sup>aX</sup>	7.9±0.9 <sup>aX</sup>
	PP	6.4±2.3 <sup>abX</sup>	5.5±0.6 <sup>bX</sup>	5.2±0.3 <sup>bcX</sup>	5.3±0.6 <sup>abX</sup>	5.7±1.7 <sup>aX</sup>
	C	6.7±1.7 <sup>abX</sup>	5.3±0.3 <sup>bX</sup>	4.8±1.3 <sup>cX</sup>	4.6±2.2 <sup>bX</sup>	4.9±0.1 <sup>aX</sup>
b Value	GT	7.2±0.4 <sup>cY</sup>	7.9±0.2 <sup>bXY</sup>	9.0±0.2 <sup>abX</sup>	9.0±0.5 <sup>aX</sup>	8.1±0.7 <sup>aXY</sup>
	GS	11.6±0.2 <sup>aX</sup>	11.3±0.8 <sup>aX</sup>	10.1±0.4 <sup>abXY</sup>	10.7±1.1 <sup>aXY</sup>	9.1±1.1 <sup>cY</sup>
	PP	9.6±0.4 <sup>bX</sup>	10.8±1.5 <sup>aX</sup>	10.2±0.9 <sup>aX</sup>	10.9±1.4 <sup>aX</sup>	10.1±1.5 <sup>aX</sup>
	C	8.5±0.6 <sup>bX</sup>	8.1±0.7 <sup>bX</sup>	8.2±0.8 <sup>bX</sup>	9.1±0.7 <sup>aX</sup>	8.9±1.0 <sup>aX</sup>

<sup>1</sup> Values are mean ± standard deviation

\* not included statistically due to high standard deviation

Means within the same column (a,b,c) and the same row (X, Y, Z) with different letters are different (P<0.05).

GT: Green tea extract, GS: Grape seed extract, PP: Pomegranate peel extract, C: Control

In this research, dry matter of green tea, grape seed and pomegranate peel extracts were determined as 53.83±0.65%, 52.93±0.67% and 52.28±0.82%, respectively. The presence of phenolic compounds in green tea extract was 2.278±1.83%, where statistically higher ( $P<0.01$ ) than the other plant extracts. Antioxidant activity was found as 1.772±0.071 mM trolox. Ivanova *et al.* (2005) reported that total phenolic material content of green tea, extracted in hot water was 317.62±3.76 µM quercetin equivalent and antioxidant activity 5.91±0.14 mM trolox (Yerlikaya and Gokoglu 2010) [48]. These parameters as 20.55±0.21 g GAE/100 g for phenolic content in microwave applied green tea, extracted in methanol and 126±4.5 mg GAE/g FRAP and 3000±778 mg AA/100 g DPPH for antioxidant activity. Total phenolic content of Greek and China green tea contents were 88.1±0.41 and 1216±32 mg GAE/cup, respectively. Also, these researchers found antioxidant activities as 0.13 mg extract/mg DPPH for Greek tea and 0.57 mg extract/mg DPPH for China tea. Total phenolic compound content and antioxidant activity of grape seed extract were found as 1.077±1.52% and 1.605±0.045 mM trolox. A positive relation was observed between phenolic content and antioxidant activity of plant (Yerlikaya and Gokoglu 2010) [48]. Existence of total phenolic compound in pomegranate peel extract was found as 1.065±0.97, and antioxidant activity was 1.784±0.033 mM trolox. In spite of containing less total phenolic content than green tea extract, pomegranate peel had similar antioxidant activity which was statistically insignificant. Plant variety, moisture of material, used solvent, applied time and temperature differences excite disparity in findings. Moreover, antioxidant activity determination method was another factor makes it difficult to compare the results. It is not appropriate to compare these data with the results of our study directly due to the differences in the reaction mechanisms of antioxidant capacity assays. First decision about food before consuming is up to its brightness (Yerlikaya and Gokoglu 2010) [48]. Bonito is a pelagic fish living close to sea surface. L values of samples did not differ in the first two months for all groups, however on the 4th and 5th months fillets treated with green tea extract showed the highest scores (Table 3). L values of fillets treated with pomegranate peel extract remained at low levels for all storage

months and significantly ( $P<0.05$ ) decreased, whereas the other groups leveled off their initial L values during frozen storage and found as insignificant in statistical evaluation (Yerlikaya and Gokoglu 2010) [48].

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