



## *The Antibacterial and Antioxidant Effect of Grape Seed and Green Tea Extracts on Durability of Tilapia*

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### ABSTRACT

**Background:** The current study tried to investigate the antioxidant and anti-bacterial effects of green tea and grape seed extract (with a volume of 2%) on durability of Tilapia packed in polyethylene bags, which were kept in cool temperature of  $4 \pm 1$  °C. **Methods:** Prepared fish were divided into 3 batches: 2 batches were treated by dipping for 30 min in ethanolic of green tea extract (2% v/v) and grape seed (2% v/v), respectively, while the third batch was dipped in distilled water as a control sample. The control and treated fish samples were analyzed for microbiological such as total volatile count and psychrotrophic count, and chemical such as thiobarbituric acid (TBA), and free fatty acid (FFA) values. The sensory characteristic was over a period of 20 days. **Results:** The results indicated that the two extracts' treatments delayed significantly ( $P < 0.05$ ). Lipid oxidation and process of spoilage in comparison with the psychrotrophic bacteria and total viable count control also remained lower than the proposed acceptable limit (7 log CFU/g). According to sensory, chemical, and microbiological analyses results, the treatment of grape seed extract had high quality and enhanced the beneficial effects on sensory characteristics in comparison with other treatments. **Conclusions:** the present study showed that the grape seed extract and green tea were very effective in extending the shelf life of Tilapia during refrigerated storage.

**Keywords:** *Tilapia; Grape seed; Green tea*

### Introduction

Today, a wide range of materials in small quantities are used as additives in food production. In addition to the anti-oxidative, antibacterial, and antifungal effects of plant essences, they also have anticancer effects (Burt, 2004). Among others, aquatic animals provide an important source of protein for the human body since they contain significant amounts of water and

fat soluble vitamins, minerals, and unsaturated fatty acids. These materials play an important role in the human nutrition. Fish meat is one of the best sources of animal protein (Ojagh *et al.*, 2010).

Damages caused by free radicals caused some diseases. Grape seed extract is rich in proanthocyanidin (PCO), a powerful antioxidant that can prevent cell destruction caused by free radicals. It also can repair and strengthen

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connective tissues and help enzyme activity (Bagchi *et al.*, 1997). Green tea is taken from the leaves of *Camellia sinensis* plant. The *catechins* in green tea are considered as a natural antioxidant and have beneficial effects on the body. After water, tea is the most common and the cheapest drink in the world which is divided into three categories: fermented (black tea), semi-fermented (Oolong), and not fermented (green tea) (Sharma *et al.*, 2005). Although the Asians are the major consumers of tea, people from around the world are adding this drink to their diet because of its effects on prevention of certain diseases (Cabrera *et al.*, 2006).

Unfortunately, little investigation has been conducted on Tilapia, so this study tried to investigate the antibacterial and antioxidant effect of grape seed extract and green tea on durability of Tilapia. The observed results in the increasing trend on some microbial, chemical, and sensory characteristics are consistent with the results observed in the some studies. (Mahmoud *et al.*, 2004, Mexis *et al.*, 2009, Ojagh *et al.*, 2010). The current study tried to investigate the antioxidant and antibacterial effects of green tea and grape seed extract (with a volume of 2%) on durability of Tilapia packed in polyethylene bags, which were kept in cool temperature of  $4 \pm 1$  °C.

## Materials and Methods

**Preparing the fish:** Fifty four farmed Tilapias with an average weight of 2,500 to 3,200 g were taken from a salt water Aquaculture Research Center in Bafq, Yazd, Iran. Samples were randomly selected from the same-size and healthy fish. After washing and gutting the fish, they were immediately placed into polystyrene boxes containing ice and quickly transferred to the laboratory. The samples were then washed with drinking water and gutted.

**Treating the samples:** The fish were saturated with 3 different materials; the first group was saturated with grape seed extract (with a concentration of 1.5% for 30 minutes); the second one with green tea extract (with a concentration of 1.5% for 30 minutes), and the third or the control

group was not saturated with any extract. After saturation and the dewatering operation, all treated fish were packed into polyethylene bags with low density and a thickness of 75 micrometers. They were then refrigerated at a temperature of 4 °C for further experiments in a two-day period. On the 0<sup>th</sup>, 2<sup>nd</sup>, 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup>, and 20<sup>th</sup> days, three fish were randomly selected from each group, later; they were examined for their quality (chemical, microbiological and sensory) parameters.

**Chemical test:** The fat amount was calculated according to the following formula (Bligh and Dyer, 1959):

Fat percentage = oil weight (g)/fish weight

FFA was used as the extracted oil to measure the fat percentage. In order to measure FFA, Eganet al.'s method was used based on the percentage of oleic acid. To do so, the following formula was employed (Egan *et al.*, 1981):

$$\text{FFA} = \frac{\frac{\text{normalized}}{10} * 28.2 * \text{Normal profit volume}}{\text{weight of oil sample}}$$

According to Eganet al.'s method, a spectrophotometer was applied for measuring thiobarbituric acid (TBA). The amount of this acid was calculated according to the following formula (Egan *et al.*, 1981):

$$\text{TBA} = \frac{50 \times (\text{sample's absorption} - \text{control's absorption})}{200}$$

**Microbial tests:** 25 cm<sup>2</sup> of the anterior portion of the fish skin was disinfected with 70% ethanol. Then the disinfected skin was removed by a septic tweezers, later, 10 g of underneath meat was removed and placed at 90 ml of 0.85% sterile physiology serum for 60 seconds in a laboratory mixer. Afterwards, the required concentrations were prepared. In every series of the experiment, three fish from each group were separately sampled. The ISO 8443 standard (2003) was used for total count of aerobic mesophilic microbes and the ISO17410 (2001) was applied for counting psychrophilic bacteria (ISO, 2001, ISO, 2003).

**Sensory evaluation:** The sensory tests of the samples were conducted through a trained team of 6 referees. Using the hedonic scale for sensory

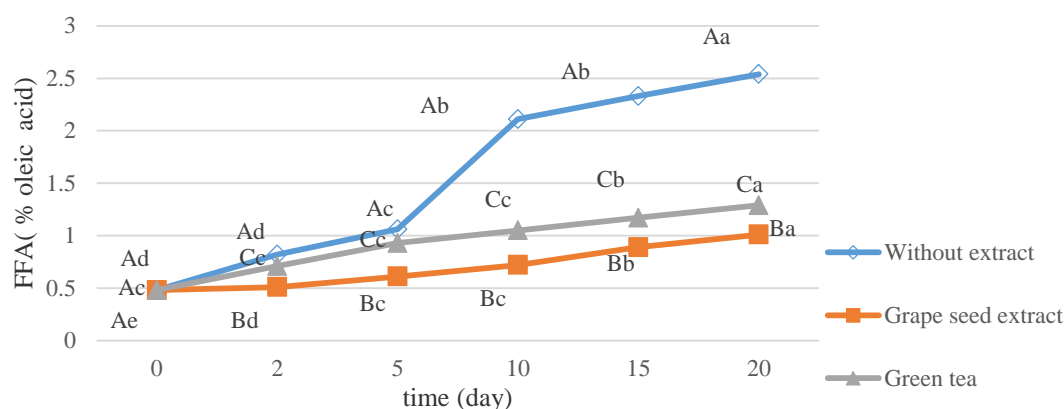
evaluation, samples were scored from 1 to 5, where (5) = very good quality, (4) = good quality, (3) = acceptable, (2) = weak, and (1) = bad quality (Twigg and Kramer, 1966). Samples were baked so that their tastes could be evaluated; 1.5% salt was added to the fish and they were steamed at 98 °C for 10-20 minutes (Ojagh et al., 2010).

**Data analysis:** The collected data were analyzed by SPSS version 20 (IBM SPSS, Tokyo, Japan) and the graphs were plotted using Excel. To accurately determine the significant difference between the treated samples, the least significant difference (LSD) was applied, further, to compare multiple means of samples, Duncan's *test* was performed.

## Results

The results of present study showed that green tea and grape seed extract treatment as well as their interactions on all studied parameters were significant.

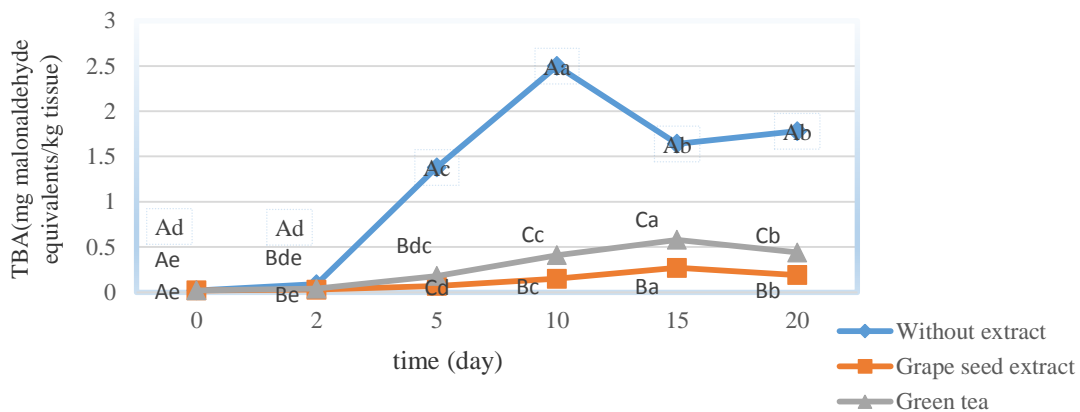
**FFA:** The FFA amount changed from the initial value of 0.48 (based on the percentage of oleic acid) to the final amount of 2.54, 1.02, and 1.21, for treatment without extract, with grape seed extract, and with green tea extracts, respectively. As it can be seen, there is a significant difference ( $P < 0.05$ ) in the results of the treated samples compared with control samples (**Figure 1**).



**Figure 1.** The amount of changes in free fatty acids (FFA) during the storage period of Tilapia. (Lowercase letters compare treatment to time, uppercase letters compare a treatment to another treatment and show significant differences at the level ( $P < 0.05$ ).

**The amount of TBA:** In this study, the amount of TBA in control samples significantly increased during the storage period compared to the treated samples. In the 10<sup>th</sup> day of storage period, this amount in the control sample reached to 2.5 mg malonaldehyde (MDA) (per kg fish tissue) which indicates a significant difference ( $P < 0.05$ )

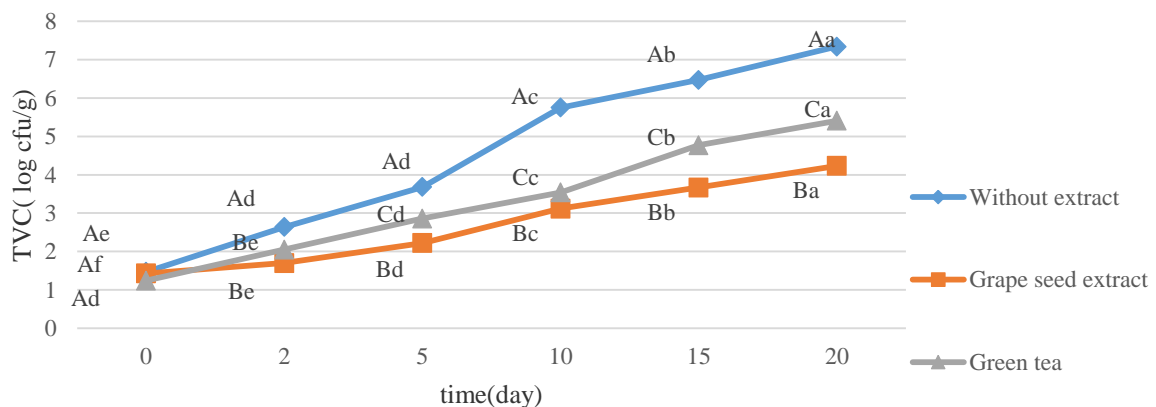
compared to samples treated with grape seed extract and green tea, with values of 0.12 and 0.37 (mg MDA equivalents/kg tissue), respectively. Then, on the 20<sup>th</sup> day, a little decrease was observed in the amount of TBA (**Figure 2**).



**Figure 2.** Changes in thiobarbituric acid (TBA) during the maintenance period (per day) of Tilapia. (Lowercase letters compare treatment to time, uppercase letters compare a treatment to another treatment and show significant differences at the level ( $P < 0.05$ ).

*Mesophilic aerobic bacteria:* In the current research, the primary total viable count (TVC) value was 1.47 log CFU/g which indicates high quality of the provided fish. According to **Figure 3**, the total amount of bacteria increased with the passage of time for all treatments so that this increase was more severe in the sample treated with no extract and its highest amount at the end of

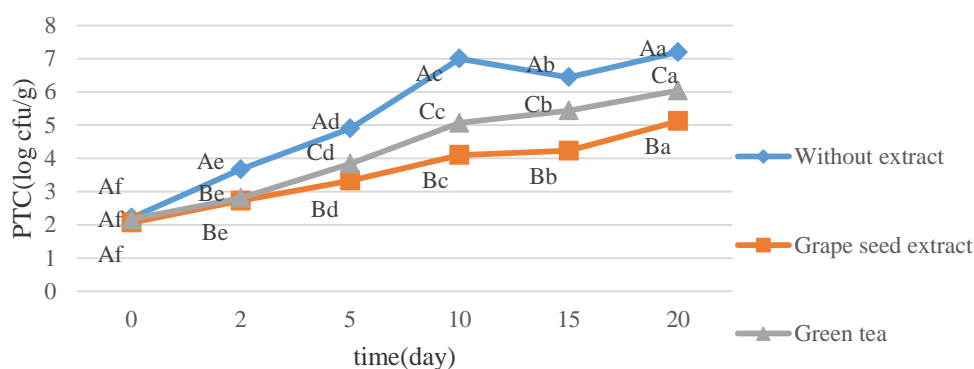
the period was 7.34 log CFU/g. This was in such a way that on the 20<sup>th</sup> day, the same index for the sample treated with grape seed was 4.231 log CFU/g and for sample treated with green tea extract was 5.29 log CFU/g; none of these two cases crossed the limit.



**Figure 3.** Total microbial load (TVC) during the maintenance period (per day). (Lowercase letters compare treatment to time, uppercase letters compare a treatment to another treatment and show significant differences at the level ( $P < 0.05$ ).

*Psychrophilic bacteria:* **Figure 4** shows that the amount of psychrophilic bacteria increases significantly by the increase in storage time ( $P < 0.05$ ). This increase in the control sample showed greater intensity, especially at the end of the maintenance period and reached the value of 7.2 log CFU/g.

**Sensory evaluation:** Results of sensory evaluation are represented in Table 1. In general, indicators of color and texture enjoyed better sustainability compared to the indicators of smell and taste.



**Figure 4.** Total psychrophilic bacteria (PTC) during the maintenance period(per day) of Tilapia. (Lowercase letters compare treatment to time, uppercase letters compare a treatment to another treatment and show significant differences at the level ( $P < 0.05$ ).

**Table 1.** Sensory evaluation results for different treatments over time

Treatments/Day	0	2	5	10	15	20
<b>Texture</b>						
Without extract	5.00 ± 0.00*	5.00 ± 0.00*	4.83 ± 0.16*	4.16 ± 0.00*	3.83 ± 0.30 <sup>b</sup>	3.33 ± 0.21 <sup>b</sup>
Grape seed extract	5.00 ± 0.00*	5.00 ± 0.00*	5.00 ± 0.00*	5.00 ± 0.00*	4.83 ± 0.16*	4.66 ± 0.21*
Green tea	5.00 ± 0.00*	5.00 ± 0.00*	5.00 ± 0.00*	4.5 ± 0.22*	4.16 ± 0.16*	3.83 ± 0.30 <sup>b</sup>
<b>Color</b>						
Without extract	5.00 ± 0.00*	4.66 ± 0.21*	4.16 ± 0.16*	4.30 ± 0.20*	3.33 ± 0.21 <sup>b</sup>	2.60 ± 0.21*
Grape seed extract	4.83 ± 0.16*	4.83 ± 0.16*	4.66 ± 0.21*	4.66 ± 0.21*	4.30 ± 0.21*	4.30 ± 0.21*
Green tea	5.00 ± 0.00*	5.00 ± 0.00*	4.30 ± 0.20*	4.30 ± 0.20*	3.83 ± 0.30 <sup>b</sup>	3.33 ± 0.21 <sup>b</sup>
<b>Smell</b>						
Without extract	4.83 ± 0.16*	4.16 ± 0.16*	4.00 ± 0.00*	3.33 ± 0.21 <sup>b</sup>	2.80 ± 0.23 <sup>b</sup>	2.00 ± 0.00 <sup>b</sup>
Grape seed extract	5.00 ± 0.00*	4.66 ± 0.08*	4.50 ± 0.34*	4.30 ± 0.20*	4.00 ± 0.28 <sup>b</sup>	3.83 ± 0.30 <sup>b</sup>
Green tea	4.83 ± 0.16*	4.50 ± 0.34*	4.00 ± 0.00*	4.00 ± 0.00*	3.66 ± 0.28 <sup>b</sup>	2.60 ± 0.21*
<b>Total Acceptance</b>						
Without extract	5.00 ± 0.00*	4.16 ± 0.16*	4.00 ± 0.00*	3.33 ± 0.21 <sup>b</sup>	3.16 ± 0.21 <sup>b</sup>	2.60 ± 0.21*
Grape seed extract	5.00 ± 0.00*	5.00 ± 0.00*	4.66 ± 0.08*	4.66 ± 0.08*	4.00 ± 0.00*	4.00 ± 0.21*
Green tea	5.00 ± 0.00*	4.66 ± 0.08*	4.66 ± 0.08*	4.30 ± 0.20*	3.83 ± 0.30 <sup>b</sup>	3.33 ± 0.21 <sup>b</sup>
<b>Taste</b>						
Without extract	4.50 ± 0.34*	4.16 ± 0.16*	3.33 ± 0.21 <sup>b</sup>	.....	.....	.....
Grape seed extract	5.00 ± 0.00*	4.83 ± 0.16*	4.00 ± 0.00*	.....	.....	.....
Green tea	4.83 ± 0.16*	4.66 ± 0.08*	4.00 ± 0.00*	.....	.....	.....

Very good quality = 5, Good quality = 4, Acceptable = 3, Weak = 2 and Bad quality = 1. Values are shown as means ± standard deviation. Within the column, values superscripted with different letters are significantly different ( $P < 0.05$ ). Values with same letters did not differ significantly ( $P > 0.05$ ).

## Discussion

The enzymes hydrolyzing fat affected the fat and consequently made major changes after the fish death and then increased the amount of FFA in them (Ojagh *et al.*, 2010, Sankar and

Raghunath, 1995). FFA is therefore a good indicator measuring the effect of lipolytic enzymes on fish fat and other meat products (Aubourg *et al.*, 2002, Dragoev *et al.*, 1998, Sankar and Raghunath, 1995). In comparing the



two samples treated with the extract, fatty acid changes in the treatment of grape seed extract was less so that no difference was observed until the 10<sup>th</sup> day ( $P < 0.05$ ), which indicates good antioxidant activity of this extract against hydrolytic spoilage. It also shows that this extract prevents the increase of FFA when compared with green tea. Özogul's study on the European also showed similar results about the increasing trend of FFA (Özogul *et al.*, 2005).

Lipid oxidation is regarded as the main reason of taste spoil (Guillén and Ruiz, 2004). In order to assess the degree of lipid oxidation in fish, TBA index was widely used. This index shows the secondary oxidation products, especially aldehydes. TBA oxidation of fats is based on the content of MDA. MDA is formed by hydroperoxide which is the outcome of the primary reactions between fatty acids and oxygen (Kostaki *et al.*, 2009). Such an increasing trend of this index is due to the increased free iron and other aldehydes peroxidation in muscle and also the production of aldehydes from the secondary products resulting from the break of the hydroperoxides.

Researchers believe that this reduction is due to the probable reaction of MDA with different compounds and existing components of muscles including proteins, amino acids, and glycogen; these reactions can cause the reduction of aldehydes. The results of measuring TBA amount in the present study was similar to that of Ojagh *et al.*'s study; they investigated the effect of trout treated with chitosan coating and also trout treated with chitosan saturated with cinnamon essence (Ojagh *et al.*, 2010).

Spoilage in fresh fish is to some extent related to the activity and growth of spoilage organisms; by producing metabolites that may lead to undesirable taste and smell of fish, they finally make them inedible (Gram and Huss, 1996).

In the present study, the lowest level of microbial load, according to **Figure 3**, was for the sample treated with grape seed extract which showed a significant difference during the whole maintenance period compared to the other investigated treatment ( $P < 0.05$ ). This fact reveals

that grape seed extract has more influence on the reduction of total bacterial load (Halawani, 2009). The observed results in the increasing trend of TVC are consistent with the results observed in the other studies (Mahmoud *et al.*, 2004, Mexis *et al.*, 2009, Ojagh *et al.*, 2010).

Psychrophilic gram-negative bacteria makes the main group of microorganisms responsible for spoilage of fresh fish that are kept cool (Gram and Huss, 1996). The allowed bacterial load for psychrotrophic aerobic cold-tolerant bacteria was reported to be 6 log CFU/g (Erkan *et al.*, 2006). Psychrotrophic bacteria, and mainly the pseudomonas species cause the increase of FFA through lipase and phospholipase enzymes (Kumudavally *et al.*, 2008).

The samples treated with green tea extract surpassed the limited value at the 20<sup>th</sup> day, while the samples treated with grape seed extract were lower than the limited value until end of the 20<sup>th</sup> day. This indicates the significant effect of the applied extract as antibacterial (Erkan *et al.*, 2006). Based on the comparison between the different treatments, the results for the least load amount of psychrophilic bacteria was related to the samples treated with grape seed extract. When compared with samples treated with green tea extract, these samples had a significant difference at the end of the maintenance period with the value of 4.98 for grape seed treatment and 5.87 log CFU/g for green tea treatment.

According to sensory analysis, shelf life of control group and both groups containing extracts, respectively 15 and 20 days. The present study showed that the grape seed extract and green tea was very effective in extending the shelf life of Tilapia during refrigerated storage.

In this study, due to lack of facilities, chemical test (PV, TVB-N) failed but good studies on shelf life of Tilapia fish was done with the help of herbal extracts.

## Conclusions

The results of chemical, microbiological, and sensory analysis of treated fish with natural

extracts during the storage period of 20 days have shown that in general, grape seed extract and green tea cause the increasing trend of measured indices to slow down in their chemical, microbial, and sensory properties. Therefore, they increase the durability of fish in the refrigerator.

The control sample exceeded the limited amount in almost all the parameters from the 6<sup>th</sup> day on. Regarding the samples treated with herbal extracts, although all three treatments were effective, the samples treated with grape seed extract showed stronger effect in chemical, microbial and, sensory indices. Consequently, they slowed down the spoilage speed of the fish so that by the end of the 20<sup>th</sup> day, none of the factors exceeded the allowed limit.

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## Author contributions

Yasini-Ardakani SA participated to conception and design of study, managing the project and drafting the manuscript. Golvarzadeh R participated to acquisition of data, data analysis and drafting the manuscript. All authors read manuscript and they finally verified it.

## Conflicts of Interest

The authors declare no conflict of interests.

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