

## Impact of Xanthan Gum Incorporated with Black Tea Extract as Edible Coating for Shelf Life Extension and Quality Maintenance of Zander Fish Fillets (*Sander lucioperca*)

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### ABSTRACT

Fish production is rising globally, aligned with issues concerning safety and quality. Therefore, it is crucial to create powerful technologies to increase the shelf life of fish. Xanthan gum is frequently utilized in the food industry to produce edible coatings. Tea has several health advantages owing to its polyphenols content which are well renowned for their antibacterial capabilities in addition to their antioxidant activity. Thus, the objective of this investigation was to elucidate the influences of xanthan gum-based edible coating (1g/l) applied alone or enriched with black tea extract (1 & 2 g/ l) as a bioactive ingredient on the biochemical, microbiological and sensory changes stored for 12 days at 4°C. Xanthan coating treatments especially with tea extract (2g/l), effectively inhibited microbial growth, delayed oxidation stress, reduced cooking loss, and retained odor, texture, and overall acceptance. The shelf life of zander fillets was extended by tea extract (2g/l) up to 9 days (based on physical attributes) and/or 12 days (based on microbial parameters) compared to the control. This study demonstrated that xanthan gum edible coating enriched with tea extract (especially with a high concentration of 2g/l) is a promising natural preservative to extend shelf-life by delaying the chemical and microbial deterioration of refrigerated zander fillets and potentially of other fish fillets.

### INTRODUCTION

*Sander lucioperca* (L.) fish species is perhaps the most well-known kind of fish preferred among consumers owing to its flavor and flesh quality (Varju *et al.*, 2018). A healthy diet for humans is advised to include fish because of its high-quality protein, fatty acid profile, and micronutrient content along with minimal levels of cholesterol, saturated fatty acids, and carbs (Michaela *et al.*, 2021). Among fish products, fish fillets have a higher nutritional content and are more important to both customers and related food sectors. They contribute roughly 16% of animal protein to human diet while also being a vital source of vitamins, trace and macro minerals, and critical fatty acids (FAO, 2016). The fish lipid fraction contains a considerable amount of n-3 polyunsaturated fatty acids, which have been shown to benefit human health by lowering the risk of cardiac and

neurologic illnesses, cancer, coronary heart disease and the advancement of coronary atherosclerosis (Mascolo *et al.*, 2013). Fish quality is regarded as the most essential characteristic since it is directly connected to customer sensory aspects such as color, consistency, smell, and flavor (Ozogul *et al.*, 2005; Hassoun and Karoui, 2017). Internal and external characteristics, such as species, original microbial load, management, processing methods, and microbial deterioration, all impact the storage life of these items (Baklari *et al.*, 2012 ; Lambrianidi *et al.*, 2019). The short shelflife of chilled seafood products is a significant issue for quality assurance and marketing profitability (Tsironi and Taoukis, 2010). Iced storage is the most frequent technique of fish preservation, especially for local consumption and short-distance transportation (Masniyom *et al.*, 2005). Fish deterioration is usually caused by autolytic processes (resulting from digestive enzymes and inherent tissue activity), bacterial activity (caused by microbial enzymes), spontaneous chemical reactions (the oxidation of lipids/discoloration), and the damage of flesh compounds (due to fish leaching from melting ice) (Giannakourou *et al.*, 2019). Pre - cooled fish products have a limited shelf life, 14-17 days at 0-4°C for an entire fish, whereas fresh fillets have a substantially shorter lifespan assuming harvesting and subsequent shipping are done properly (Aponte *et al.*, 2018). As a result, the invention and research of effective strategies are required in order to reduce economic losses resulting from fish deterioration.

Natural edible films and coatings have also been utilized to delay rotting and enhance seafood shelf life. Non-toxic and biocompatible edible films and coatings can be utilized to offer a physical barrier to protect and improve the quality of food items by slowing lipid oxidation and reducing protein and moisture loss. They can also be used as a carrier for food additives with antioxidant and antibacterial properties (Jiang *et al.*, 2019; Koc *et al.*, 2020 and Karsli *et al.*, 2021). Xanthan gum is a polysaccharide produced by plant - pathogenic bacteria belonging to the genus *Xanthomonas* (Sutherland, 1993). In the food and pharmaceutical industries, xanthan is used for antioxidant, antibacterial, nutritional and biofilm inhibitor purposes (Munir *et al.*, 2017). Natural bioactive substances are quite a useful technique for improving coating performance and quality (Xiong *et al.*, 2020).

Recently, a series of studies were conducted on the utilization of natural extracts to extend the shelf life of sea foods (Yuan *et al.*, 2016). Tea is notably high in polyphenols, such as catechins, thearubigins and theaflavins which are considered to contribute to the health advantages of tea. Apart from antioxidant activity tea polyphenols are known for their antimicrobial properties (Li *et al.*, 2012). The application of xanthan gum with black tea extract as an edible coating on the quality of refrigerated zander fillets is scarce. Thus, the objective of this study is to evaluate the effects of xanthan gum and black tea extracts on shelf life, biochemical composition and microbiological quality of refrigerated zander fillets for 3,6,9 and 12 days.

## MATERIALS AND METHODS

### 1- Fish fillets

Fresh fillets of zander fish (10 Kg) were procured from a fishing market in Cairo, Egypt. The fish fillets were directly transferred refrigerated to the laboratory.

### 2- Preparation of tea extract

Dried black tea was prepared by maceration using ethanol (1:10 w/v) and kept overnight then the mixture was centrifuged and filtered using Whatman no. 1 filter paper. The extract was concentrated under vacuum by a rotary evaporator then the obtained dried extract was kept at 4°C until further use.

#### a. Preparation of edible coating solution

Four different treatments were employed for coating fillets as follows: (1) C: control, (2) XG: 10g/L xanthan gum, (3) XG + TE1: 10g/L xanthan gum and 1.0g/L tea extract and (4) XG + TE2: 10g/L xanthan gum and 2.0g/L tea extract. Xanthan gum solution was prepared according to the method of **Quoc *et al.*, (2016)** by mixing 10g xanthan gum powder with 1L of distilled water and 10g citric acid and 5g glycerol at 85° C under magnetic stirring. Then tea extract was added at the corresponding concentration for each treatment. The solutions were stirred for about 2h.

#### b. Fish fillets coating

Fillets were cut into cubes (5x5x3 cm) and were assigned randomly into four groups. For coated treatments, fillets cubes were submerged in the corresponding coating solution for 30 min. After being withdrawn from the solution, the samples were air dried for 5 minutes at room temperature before being put in separate Petri dishes with absorbent pads below and sealed thereafter in plastic bags. The control group was uncoated and handled in the same manner as the coated groups. All treatments were kept in the fridge at 4°C. Three plates were randomly selected from each treatment group and evaluated after 0, 3, 6, 9, and 12 days.

#### c. Determination of cooking loss and pH

Zander fillets were chopped into 1cm<sup>3</sup> pieces for the cooking loss and cooked in a water bath for 15 minutes at 85 C before cooling at room temperature. The following formula was used to compute the percentage of cooking loss:  $[(W_0 - W_f) / W_0] \times 100$ , where  $W_0$  and  $W_f$  were the fillets' initial and final sample weights before and after cooking, respectively (**Feng *et al.*, 2016 and Karsli *et al.*, 2021**). For pH determination, 10g of zander fillets were crushed and homogenized with 100 ml of deionized water for 60s. The pH value was recorded using a digital pH meter (HANNA).

#### d. Proximate composition

Moisture content was determined after drying the fillets samples to constant weight in an oven at 105° C (**AOAC, 2019**). Total protein content was determined by Kjeldahl procedure for total nitrogen and evaluated by multiplying by a factor of 6.25 (**AOAC 2019**). Lipid content was obtained by soxhlet method. Ash was determined in muffle at 600°C according to **AOAC (2019)**.

#### e. TBA

Thiobarbituric acid (TBA) was determined using spectrophotometric ally according to the procedure of **Tarladgis *et al.* (1960)**.

#### f. Free fatty acids

The free fatty acids were measured using the technique described by **Bernardez *et al.*, (2005)**. 50 mg of each sample were homogenized with cyclohexane and cupric acetate-pyridine reagent, vortexed for 2 minutes, and then centrifuged at 9000 rpm for 20 minutes. The activity was identified at 710nm.

#### g. Total phenols and antioxidant activity

The total phenolic compounds in the various samples were measured using the Folin-Ciocalteu technique (**Singleton *et al.*, 1999**) with gallic acid as the standard. The findings were represented as milligramme gallic acid equivalent per 100 millilitres (or milligrammes) (mg GAE/100 ml (mg)). The total antioxidant capacity of the samples was evaluated using the phosphomolebdenum technique with ascorbic acid as the standard (**Prieto *et al.*, 1999**). The results were represented in milligrammes of ascorbic acid equivalent per 100 millilitres (or milligrammes) (mg AAE / 100 ml (mg)).

#### h. Microbiological analysis

Fish samples were subjected to microbiological analysis to determine Microbiological analysis (total bacterial counts, total yeasts, total fungi, *salmonella*, *Escherichia coli*, *Staphylococcus aerus*, *Bacillus cereus*) of the fish samples was performed using standard methods of **APHA (2005)**.

#### i. Physical attributes

The physical attributes of raw fish was examined by a number of 10 panelists. Appearance, texture and odor of raw fish were evaluated. Rating was assigned separately for each parameter on a 1 to 10.

#### j. Statistical analysis

The statistical analysis was performed according to **Snedecor and Cochran (1980)** using (ANOVA), while the least significant difference procedure was used to test the difference between means significance that was defined at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Effect of different treatments and storage periods on fish fillets proximate composition

Table (1) shows the results for the effect of different coating on the moisture, protein, lipids, carbohydrates and ash of fish fillets at different storage days. The data revealed that the edible coatings as well as the storage period have significant ( $p < 0.05$ ) effects on the moisture and lipid content of fish fillets. On the other hand, the protein and carbohydrates content of fish fillets were not significantly ( $p > 0.05$ ) affected neither by the edible coating film nor by the storage period. The ash percentage of fish fillets was

significantly ( $p < 0.05$ ) affected by the edible coating used while the storage period did not show any significant ( $p > 0.05$ ) effect on the ash content of fillets samples.

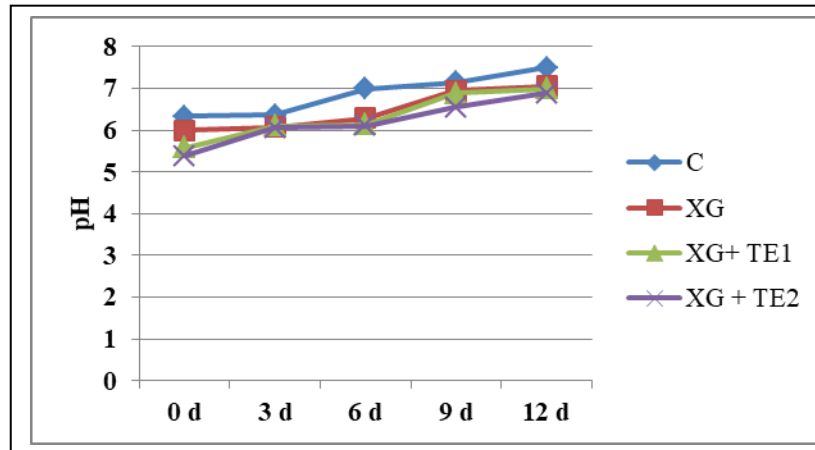
**Table (1): Proximate analysis (fresh weight)**

Storage days (SD)	Edible coating (EC)	Moisture	Protein	Lipids	Carbohydrates	Ash
0 d	C	73.16	14.25	0.24	11.64	0.71
	XG	76.10	15.99	0.20	7.09	0.62
	XG + TE1	77.92	21.41	0.17	0.04	0.46
	XG + TE2	78.36	17.53	0.19	3.43	0.49
3 d	C	70.82	14.02	0.31	13.90	0.95
	XG	73.92	15.64	0.25	9.48	0.71
	XG + TE1	76.37	21.04	0.31	1.64	0.64
	XG + TE2	77.00	17.36	0.28	4.78	0.58
6 d	C	69.36	13.79	0.54	15.20	1.11
	XG	72.49	15.13	0.22	11.41	0.75
	XG + TE1	75.20	20.61	0.26	3.28	0.65
	XG + TE2	75.89	17.29	0.22	5.97	0.63
9 d	C	67.56	12.91	0.77	17.58	1.18
	XG	71.71	14.23	0.44	12.80	0.82
	XG + TE1	74.59	19.61	0.62	4.52	0.66
	XG + TE2	75.29	16.58	0.71	6.76	0.66
12 d	C	66.90	11.84	0.80	19.24	1.22
	XG	71.17	12.59	0.59	14.75	0.90
	XG + TE1	74.18	17.06	1.19	6.87	0.70
	XG + TE2	75.00	13.95	0.80	9.58	0.67
<b>LSD(0.05)</b>	<b>SD</b>	<b>0.049</b>	<b>0.080</b>	<b>0.031</b>	<b>0.120</b>	<b>0.053</b>
	<b>EC</b>	<b>0.044</b>	<b>0.072</b>	<b>0.027</b>	<b>0.107</b>	<b>0.048</b>
	<b>SD x EC</b>	<b>0.098</b>	<b>0.161</b>	<b>0.062</b>	<b>0.240</b>	<b>0.107</b>

### Effect of different treatments and storage periods on fish fillets pH

Figure (1) shows the pH variation of fish fillets samples stored at 4°C for twelve days. According to the findings, the pH of the control samples augmented from 6.33 to 8.00 after 12 days of refrigerated storage. Also the pH value for the fillets of treated groups increased over storage period but the recorded values for treated groups were lower than those of control. The pH values for XG, XG+TE1 and XG+ TE2 samples after 12 days of storage were 7.35, 7.10, and 6.38, respectively. In general, it could be concluded that as the storage time lengthened, the pH values of coated and uncoated zander fillets. At the completion of the storage period, the rise in pH values of the non-coated samples (control) was more noticeable. According to **El Sheikha et al., (2022)** this may take place as a result of the pH rising due to the buildup of ammonia and amino acid breakdown products. Due to a rise in protease activity or microbial growth, a rise in

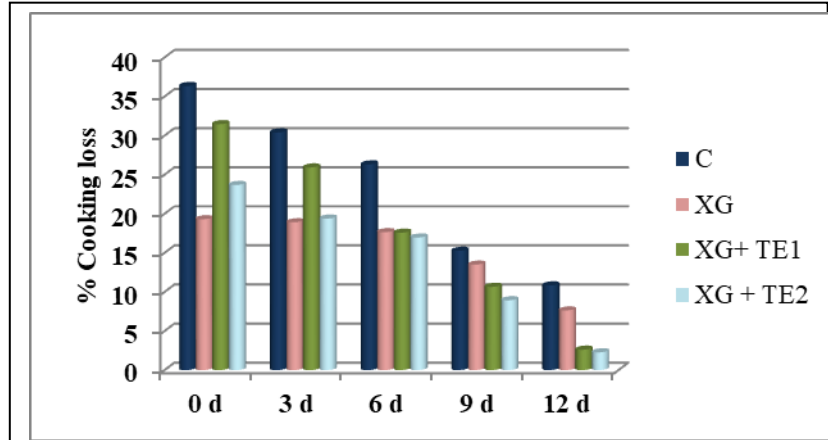
the pH of preserved fish may be associated with the formation of amino acids, peptides and ammonia.



**Fig. 1. Effect of different treatments and storage periods on fish fillet pH**

#### **Effect of different treatments and storage periods on fish fillet cooking loss**

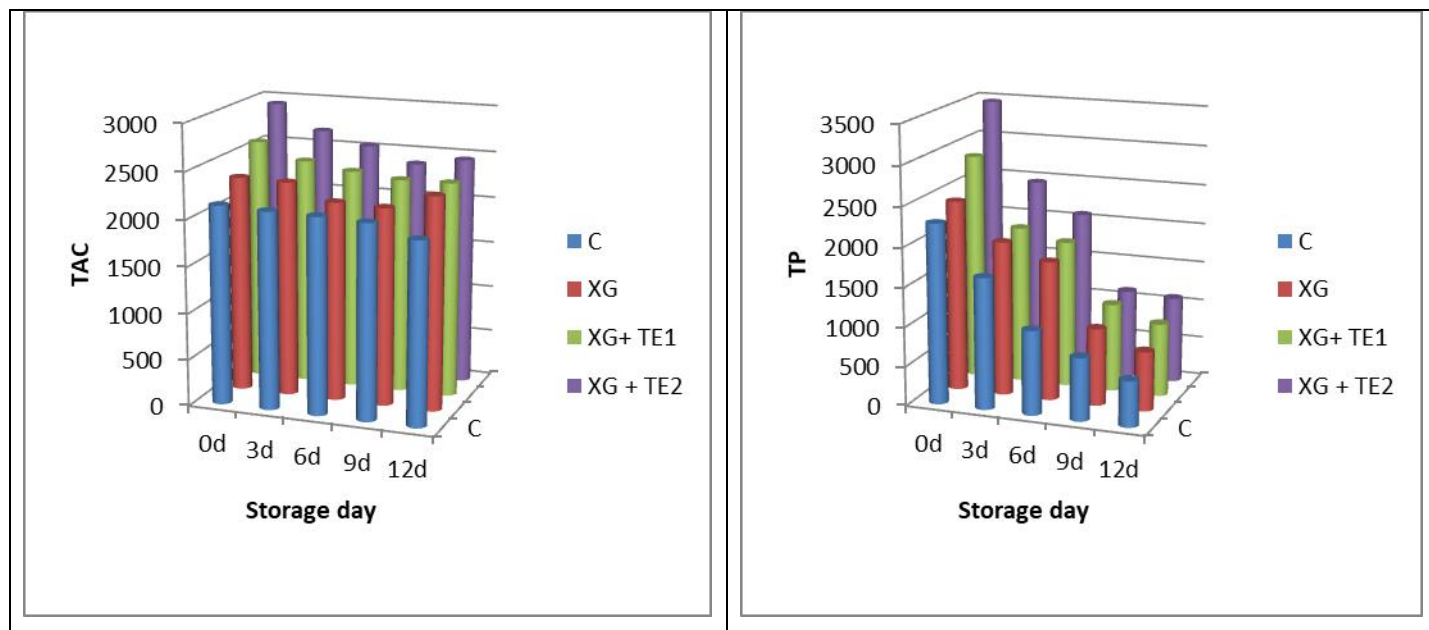
Cooking loss is an additional vital factor for judging the quality of meat products after cooking and is often linked through the requirement for both water and lipids in proteins (Sayas-Barbera *et al.*, 2011). High cooking loss in frozen foods is undesirable and may be a sign that the quality is deteriorating because to water exudation. The cooking loss of coated (XG, XG+TE1, and XG+TE2) and uncoated fish fillets during the 12 days of storage at 4 °C, is shown in figure 2. The data show that the values of cooking loss for coated and uncoated samples were initially high at the start of the storage period and then started to decline with increasing the storage period up to 12 days. The data also indicate that during the different storage days, the cooking loss values of uncoated samples were higher than those of all coated samples. According to Cao *et al.*, (2016), there have been very few papers that discuss how cook loss is affected by cold storage temperatures before heat treatments. In one study, it was found that the cooking loss of black carp fillets kept at 4°C for up to 12 days fluctuated with the length of storage period. While another study showed that the cook loss of silver carp held on ice for up to 3 days before thermal treatments tended to rise with the amount of time spent storing the fish. The heat loss of fish muscle did, however, reduce with ice storage duration for up to 9 days in another investigation on bighead carp fish muscle but subsequently it rose until the 21<sup>st</sup> day.



**Fig. 2. Effect of different treatments and storage periods on fish fillet cooking loss**

### **Effect of different treatments and storage periods on fish fillet antioxidant capacity and phenolics' content**

The findings of the present investigation for the antioxidant activity in fish fillet sample are displayed in Figure 3a. Figure 3a shows that throughout the storage period, antioxidant activity significantly decreases in all treatments ( $p < 0.05$ ). A similar pattern of change was seen in total phenolic content concentration of fillets samples during storage as shown in fig. 3b. The results show that the decrease in antioxidant activity was higher in the control (uncoated) samples compared to the coated treatments. According to **Salsabiela *et al.*, (2022)** antioxidant molecules are affected by several factors and because tea is high in antioxidants, the antioxidant activity of the samples can be enhanced by including black tea extract to the active coating solution. Also the presence of xanthan in the edible coat can help to limit the loss of antioxidant activity during storage. This is due to the fact that edible coatings operate as a shield, blocking oxygen and moisture out of the enzymatic oxidation of phenolic compounds (**Khodaei *et al.*, 2021**).



**Fig. 3 a, b. Effect of different treatments and storage periods on fish fillet (a) antioxidant capacity and (b) total phenolic compounds**

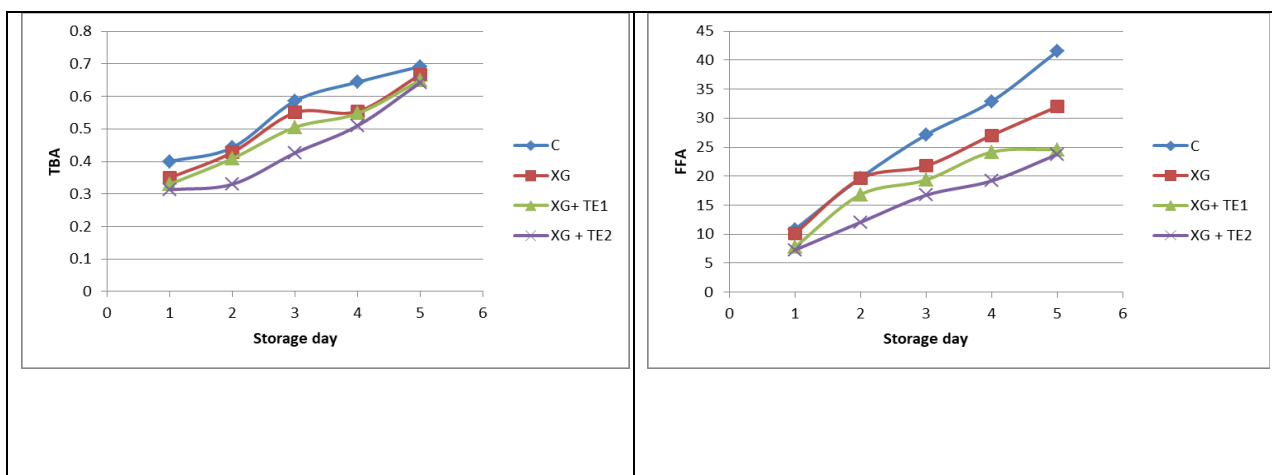
#### Effect of different treatments and storage periods on fish fillet TBA and FFA

The thiobarbituric acid values (mgMDA/kg) of control and treated samples during storage are illustrated in Fig. (4a). The TBA recorded values of the control, XG, XG+TE1 and XG+TE2 at zero time were 0.400, 0.350, 0.330 and 0.313 mgMDA/kg, respectively; while they significantly increased after 12 days of storage (0.692, 0.667, 0.650 and 0.643 mgMDA/kg, respectively). It can be observed that during storage, the TBA values of all treatments increased with increasing the storage period. This rise in TBA value during refrigerated storage might be ascribed to partial dryness of the fish and enhanced oxidation of unsaturated fatty acids (Valipour Kootenaie *et al.*, 2017). The degree of lipid oxidation, notably in meat and fish, is determined by the TBA value, which also serves as a fish quality indicator. When peroxides are oxidized to produce aldehydes and ketones, the secondary oxidation product, MDA, is used to compute TBA (Karimzadeh, 2022). According to Ehsani *et al.*, (2012), it has been observed that the maximal TBA value indicating satisfactory fish quality during storage is 1-2 mg MDA per kilogram lipid. Thus it can be concluded that during the present study, at the end of the storage period, the final levels of TBA were within the allowable concentrations.

The formation of free fatty acids is induced by lipid hydrolysis. As a result in fig. b, measuring the FFAs % can be utilized advantageously as an indication of the degree of lipolysis, which is an indication of fish freshness (Valipour Kootenaie *et al.*, 2017). Free fatty acid (FFA) formation as a result of enzymatic and non-enzymatic lipid hydrolysis is employed as a lipid quality indicator. FFA production is frequently caused by endogenous enzyme catalysis (Ehsani *et al.*, 2012). In the present work, the free fatty



acid content of fish fillets samples gradually increased along the 12 days of storage. Also, the results revealed that the values of FFA recorded for the control (uncoated) fish fillets samples were higher than those for treated (coated) samples indicating that the edible coatings applied have a good effect on preserving fish fillets quality. These findings are in agreement with previous studies which reported that the application of active edible coatings to fish fillets was able to protect fresh trout fillets against lipid oxidation for up to 15 days (Volpe *et al.*, 2015). Also, similar trend was reported for other fish species such as Rainbow trout, Beluga sturgeon, carp and mackerel (Dragoev, 2008; Hosseini *et al.*, 2010; Ojagh *et al.*, 2010).

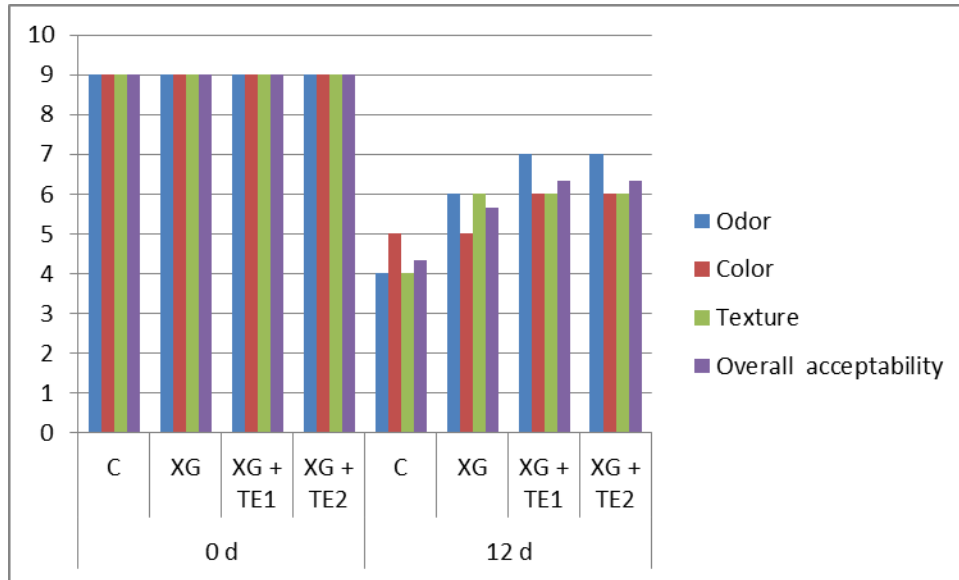


**Fig. 4 a, b. Effect of different treatments and storage periods on fish fillet TBA and FFA**

### Physical quality of fillets

The physical quality of zander fillets during storage was evaluated by examining their color, odor, texture as well as general consumer acceptability. At the start of the storage period, all the fillets samples had a fresh fish smell and a distinct shiny surface with the normal texture usually monitored by consumers. The overall acceptability of the samples in all groups exceeded 9 on a scale of 10. On the other hand, as the storage started, the fillets samples begin to lose their physical characteristics and this loss was significantly faster in the control fillet samples (uncoated) compared to the coated samples. According to **Gharibzahedi and Mohammadnabi (2017)**, the edible coatings can significantly preserve the color of fish cuts during cold storage by slowing oxidative lipid degradation. The same authors also reported that the improved texture preservation during refrigerated storage with edible coating encapsulated with natural extract may be attributable to a delay in the degradation and disintegration of myofibrillar and collagen proteins caused by a decrease in the activity of endogenous enzymes and microbes in fish fillets (**Gharibzahedi and Mohammadnabi, 2017**). As a result, in our current investigation it could be concluded that the use of tea extract (with its proved antioxidant

and antimicrobial activities) along with xanthan gum in the edible coating led to preserving zander fillets physical quality during refrigerated storage.



**Fig. 5. Physical quality of fillets**

#### **Microbiological properties of fish fillets**

Table (2) presents the results for the effect of different coating films on the total bacterial count, total fungi, total yeast, *E.coli*, salmonella, *Bacillus cereus* and staphylococcus during storage for 12 days.

Even though it is often believed that freshly caught fish are devoid of bacteria, studies have shown that a variety of fish species contain a variety of bacteria that, in some circumstances, have the potential to be pathogenic, including *staphylococcus* and *pseudomonas angulluseptica* (Giddings *et al.*, 2015).

In order to maintain the nutritional value and quality of fish while preventing waste and losses, post-harvest handling, processing, preservation, packaging, storage, and transportation must be done with special care. Fish can be supplied and marketed globally in a variety of product forms intended for food or non-food uses, from live organisms to more complicated preparations, thanks to preservation and processing that can lower the rate of deterioration (Viale delle, 2006).

**Table 2. Microbiological properties of fish fillets**

	<b>Zero time</b>	<b>3days</b>	<b>6 days</b>	<b>9 days</b>	<b>12 days</b>
<b>Total bacterial count CFU/g</b>					
<b>Control</b>	25 x10 <sup>3</sup>	20 x10 <sup>4</sup>	33 x10 <sup>6</sup>	55 x10 <sup>7</sup>	83 x10 <sup>8</sup>
<b>Xanthan</b>	15 x10 <sup>3</sup>	20 x10 <sup>4</sup>	80 x10 <sup>4</sup>	53 x10 <sup>5</sup>	31 x10 <sup>6</sup>
<b>Xanthan 1000</b>	15 x10 <sup>3</sup>	12 x10 <sup>3</sup>	32 x10 <sup>4</sup>	50 x10 <sup>4</sup>	30 x10 <sup>5</sup>
<b>Xanthan 2000</b>	10 x10 <sup>3</sup>	30 x10 <sup>3</sup>	50 x10 <sup>3</sup>	28 x10 <sup>4</sup>	80 x10 <sup>4</sup>
<b>Total fungal count CFU/g</b>					
<b>Control</b>	ND	ND	ND	ND	ND
<b>Xanthan</b>	ND	ND	ND	ND	ND
<b>Xanthan 1000</b>	ND	ND	ND	ND	ND
<b>Xanthan 2000</b>	ND	ND	ND	ND	ND
<b>Total yeast and molds count CFU/g</b>					
<b>Control</b>	1.2x10 <sup>2</sup>	1.5x10 <sup>3</sup>	6.6x10 <sup>3</sup>	9.0x10 <sup>4</sup>	12 x10 <sup>4</sup>
<b>Xanthan</b>	1.35x10 <sup>2</sup>	3.0x10 <sup>3</sup>	4x10 <sup>3</sup>	8x10 <sup>3</sup>	10 x10 <sup>3</sup>
<b>Xanthan 1000</b>	1.44 x10 <sup>2</sup>	2.2 x10 <sup>2</sup>	5.2x10 <sup>2</sup>	8 x10 <sup>2</sup>	22 x10 <sup>2</sup>
<b>Xanthan 2000</b>	1.23 x10 <sup>2</sup>	3.2 x10 <sup>2</sup>	4.5 x10 <sup>2</sup>	5.7 x10 <sup>2</sup>	6.6 x10 <sup>2</sup>
<b>Salmonella count CFU/g</b>					
<b>Control</b>	ND	ND	ND	ND	ND
<b>Xanthan</b>	ND	ND	ND	ND	ND
<b>Xanthan 1000</b>	ND	ND	ND	ND	ND
<b>Xanthan 2000</b>	ND	ND	ND	ND	ND
<b><i>E.coli</i> O<sub>157</sub> count CFU/g</b>					
<b>Control</b>	ND	ND	ND	ND	ND
<b>Xanthan</b>	ND	ND	ND	ND	ND
<b>Xanthan 1000</b>	ND	ND	ND	ND	ND
<b>Xanthan 2000</b>	ND	ND	ND	ND	ND
<b><i>Bacillus cereus</i> count CFU/g</b>					
<b>Control</b>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>
<b>Xanthan</b>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>
<b>Xanthan 1000</b>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>
<b>Xanthan 2000</b>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>	<10 <sup>3</sup>
<b><i>Staphylococcus</i> count CFU/g</b>					
<b>Control</b>	< 100	< 100	< 100	< 100	< 100
<b>Xanthan</b>	< 100	< 100	< 100	< 100	< 100
<b>Xanthan 1000</b>	< 100	< 100	< 100	< 100	< 100
<b>Xanthan 2000</b>	< 100	< 100	< 100	< 100	< 100

The results of the total bacterial count shown in Table 2 are represented by colony forming unit per gram. It was observed that as the storage duration was extended, the microbial count of all samples analyzed tended to steadily grow. The highest number of bacterial count was obtained from uncoated samples (control) at the end of storage period (12 days) which was  $(83 \times 10^8 \text{ cfu/g})$  followed by xanthan then xanthan plus TE1. On the other hand, the lowest number of bacterial count was obtained from xanthan + TE2 and it was  $80 \times 10^4 \text{ cfu/g}$ . These results indicate that the coating films prevented the increase in microbial count during the cooled storage. Generally, the addition of black tea extract to xanthan strengthened its antimicrobial activity towards the microbes. Low microbial-load indicates high-quality of fish and fish products.

All tested samples were lower than the maximum permissible levels (MPLs) recommended by the International Commission for microbiological specifications of total bacterial count in fish and fish products which is below  $10^7$  counts cfu/g. till the last period of chilled storage while uncoated samples (control) was acceptable till the day 6 of chilled storage then it exceeded the limit of  $>10^7$  counts (rejected) which was  $55 \times 10^7$  at day 9 and  $83 \times 10^8$  at day 12.

According to the International Commission on Microbiological Specifications for Foods (ICMSF), the total bacterial count is a significant consideration when evaluating the level of microbial contamination in food products (**ICMSF, 2010**). The quantity of microorganisms is estimated in order to determine the quality, shelf life, and safety of food. Plate counts below  $5 \times 10^5$  are seen as being of high quality, between  $5 \times 10^5$  and  $10^7$  is regarded as being of moderately acceptable quality, and plate counts  $10^7$  are regarded as being of unacceptable quality with representative sample units of at least five (**Alkuraieef *et al.*, 2022**).

Inadequate sanitation practises during fish harvesting, handling, production, storage, shipping, and marketing may be to blame for the fungus contamination of fish (**Arafa *et al.*, 2021**). Fish that has been contaminated by fungi are thought to be the primary reasons behind the bad flavours and unappealing tastes that indicate rotting and may pose a risk to public health in addition to causing significant financial losses (**Hassan *et al.*, 2011**). Due of odd flavours, sliminess, lipolysis, and unappealing taste, which render the product of inferior quality unmarketable or even unfit for human consumption, fungal spoilage typically only counts these organisms when a problem occurs. No fungal contamination detected in our treatments to the end period of chilled storage, it means that the samples were completely save to consumption by human.

In addition to bacteria, several genera of yeasts, mainly *Rhodotorula*, *Torulopsis* and *Candida*, may also be present in small numbers among the surface microflora of fish. Although yeasts are quite common in both fresh and salt water, there is very little research on their presence in living fish (**Lougovois and Kyrana, 2014**). Due to the

speed at which fish may deteriorate, The most frequent microbial deterioration culprits in fish that has been chilled are yeast and mould species. The obtained results that are found in Table (2) showed that the yeast and mold were gradually increased in all samples throughout storage time until the end storage. Zander fillets had a main yeast/mold count between 1.2 and  $1.44 \times 10^2$  CFU/g on day 0. The findings of the current study revealed that all treatments containing xanthan and those containing xanthan + tea extract had the capacity to control the growth of yeasts and moulds relative to the untreated fillet samples (control) when cooling. These results were in agreement with **Viegas *et al.*, (2013)** and **El-Sherif *et al.*, (2021)**.

It can be said that the qualities of zander fillets are preserved for a longer period of time when stored under refrigeration owing to the coating of xanthan and ethanolic black tea extract. As compared to the uncoated fillet samples, the shelf life of zander fillets increased by roughly 12 days (control). Because it is safer and biodegradable than synthetic gelling agents, xanthan gum offers advantages over them; this conclusion was in agreement with **El Sheikha *et al.*, (2022)**.

Salmonella and other infections have raised worries, thus the FDA has increased the importance of inspection. According to the findings listed in table (2), no Salmonella strains were found in the samples that were examined for this investigation, which was consistent with earlier research on seafood items. While on the other side, seafood goods, ready-to-eat products, cooked crab, dried/salted seafood, smoked seafood, and prepared foods were all found in 11 of 228 (4.8%) samples (**Heinitz *et al.*, 2000**).

Without the right sterilisation process, Coliform (including *E. coli*) is a bacteria that can cause food contamination and illness that affects the digestive system. Fish and fish products containing the coliform group of bacteria, primarily Citrobacter, Enterobacter, Escherichia, and Klebsiella, pose a health risk to people (**Sheraa, 2018**). *E. coli* O<sub>157</sub> could not be isolated from any of the fish samples used in this study. The worldwide committee on microbiological standards for food specifies that the maximum permissible levels of total coliforms (TC) for fresh and frozen fish are 100 MPN/g. It implies that our samples are entirely safe for eating by humans.

Results in table showed that *Bacillus cereus* count in all samples control, xanthan, xanthan + TE1 and xanthan + TE2 don't exceed  $10^3$ . This result agreed with **Hassanien *et al.*, 2018** who reported that Raw or cooked fish may be harbored *B. cereus* which can cause human illness due to it characterized by spore formation and can produce two types of toxin; diarrhetic and emetic toxins (**Hassanien *et al.*, 2018**). Also the result agreed with **Granum and Lund, (1997)** who came to the conclusion that there are  $10^5$ – $10^8$  live cells or spores in the entire infective dose. As a result, no meal that contains more than  $10^3$  *B. cereus*/g may be deemed 100 percent safe for consumption. It is indicate that our

treatment used to preserve fish fillet under chilled condition allow fish fillet to be completely save for consumption.

Food that has *Staphylococcus arueus* in it has likely been contaminated through the skin, mouth, and/or nose of those who handled it. Equipment that hasn't been properly cleaned might be a source of infection. *S.aureus* was isolated from employees at fish processing plants and goods derived from fisheries. Small amounts of these bacteria in fisheries products are not a severe issue, but if the product is handled carelessly during processing, leading to excessive multiplication, food poisoning may proceed ( **Hassanien *et al.*, 2017**).

In our research data showed that the number of *Staphylococcus arueus* in all fillets sample were acceptable according to International Commission for microbiological specifications of food these results in agreement with **Edris *et al.* (2017)**. It is advised to use sanitary gloves when handling ready-to-eat meals to reduce the problem of *S. aureus* contamination because the presence of this organism shows that hygienic conditions were not maintained throughout processing and storage (**Hussein Ali, 2014**). On the other hand these results didn't agreed with **Arafa *et al.*, (2021)** who detected *S. aureus* in high mortality rates in freshwater aquaculture.

From the microbiology results, it can be concluded that shelf life of zander fillets could be extended by coating using Xanthan + tea extract for a period up to 12 days without microbial deterioration. Other studies suggesting similar results for other fish species and other edible coatings are summarized in table (3).

**Table (3): Comparison of different coatings and fish species based on previous studies**

<b>Fish</b>	<b>Edible coating</b>	<b>Days of storage</b>	<b>Reference</b>
Salmon	xanthan gum-Litsea cubeba essential oil nanoliposome	8	(Cui <i>et al.</i> , 2022)
Mackerel Tuna	xanthan gum- Propolis	20	(El Sheikha <i>et al.</i> , 2022)
Shrimp	Lepidium sativum seed gum-carvacrol	18	(Karamkhani <i>et al.</i> , 2018)
Beluga sturgeon	Jujube gum - nanoemulsions nettle essential oil	15	(Gharibzahedi and Mohammadnabi, 2017)
bream (Megalobrama amblycephala)	alginate-based Vitamin C and tea polyphenols	21	(Song <i>et al.</i> , 2011)
Rainbow trout (Oncorhynchus mykiss)	Salep gum containing concentrations orange peel essential oil	16	(Agdar <i>et al.</i> , 2021)

## CONCLUSION

Promising results were obtained from the present work regarding the use of edible coatings containing xanthan and black tea extract for preserving the quality of zander fish fillets over twelve days of refrigerated storage. The results indicated that an edible coating containing xanthan gum incorporated with 2g/l of black tea extract was highly efficient in delaying the oxidative damage of fish fillets with preserving their safety by inhibiting microbial growth. We can recommend employing edible coatings containing xanthan gum enriched with black tea extract as a powerful, safe, natural, and less expensive substitute to artificial preservatives for the production of active coatings for preserving zander fish fillets. Future studies could be addressed to identify the effect of these kinds of coatings on other fish species and fishery products.

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