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Effectiveness of some Cryoprotectant Agents on Chemical Composition, Physicochemical, Microbiological and Sensory Quality Criteria of some Marine Fish Species

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ABSTRACT

The effects of some cryoprotectants agents using sodium hexametaphosphate (SHMP), sodium tripolyphosphate (STPP), chitosan and xanthan on the chemical composition, physicochemical, microbiological and sensory quality properties of some marine fish species namely threadfin bream (*Nemipterus furcosus*), goldband goatfish (*Upeneus moluccensis*), marbled spinefoot (*Siganus rivulatus*) and gilt-head sea bream (*Sparus aurata*) collected from Mediterranean Sea during December 2020 were examined. The results showed that, the moisture, protein, lipid and ash of threadfin breams, goldband goatfish, marblede spinefoot and gilt-head sea bream fish species were (77.16, 75.13, 78.09, 76.81%); (19.68, 19.90, 18.08, 19.71%); (2.25, 2.45, 2.77, 2.52%) and (1.38, 1.65, 1.78, 1.08%), respectively. While the pH, TVB-N, TMA and TBA values of the examined fish species were ranged from (6.05 to 6.60); (15.12 to 19.35, mg/100g); (0.45 to 0.68 mg/100g) and (0.48 to 0.82 mg MDA/kg), respectively. Total bacterial count and coliforms count values of the investigated fish species ranged from (5.214 to 5.701 log_{10} cfu/g) and (3.00 to 4.477 log_{10} cfu/g), respectively. The sensory properties of the treated fish samples were better than control group. In conclusion, cryoprotectants agents had a clear effects for improving the physicochemical, microbiological and sensory properties of the studied fish samples while, it had no differences in chemical composition. On the other hand, polyphosphate was the most effective agent followed by chitosan and xanthan, so phosphate treatment can be an alternative way to improve the quality of some marine fish species.

Keywords: Seafood, cryoprotectant, chemical composition, physicochemical, sensory evaluation

INTRODUCTION

Phosphate is used in the food industry to improve sensory properties such as texture and the ability to bind water due to the high efficiency of phosphorous in increasing the functional properties of processed food products as a basic alternative to sodium chloride salt (Robe & Xiong, 1992). Therefore, in order to produce high nutritional value fish products, a good understanding the mechanism of interaction of phosphate with myofibril protein is necessary (Masniyom, *et al.*, 2005). Phosphate is also used in the manufacture of meat and fish as one of the legally permitted additives to improve the functional quality characteristics (Trout & Schmidt, (1984). Polyphosphate is the association of a number of simple phosphate units with each other and is often found in all living organisms as it is an essential component of our food system. Nowadays, many food phosphates additives have used to improve their functional quality characteristics such as sodium tripolyphosphate (STPP) tetrasodium pyrophosphate (TSPP), sodium hexametaphosphate (SHMP), in different meat and fish products such as sausage, burger, fingers, balls, ect. (Huang, *et al.*, 2019 and Thangavelu, *et al.*, 2019). Chitosan has many biological and functional activities including: Antimicrobial activity against bacteria, fungi and yeast, antitumor, lowering cholesterol level in blood, water, fat and dye binding abilities, emulsification, gel formation, chelating of metal ions, edible film-forming property and antioxidant activity. Applications of chitosan in fields of food processing, nutrition, chemical engineering, pharmaceuticals and environmental protection have received considerable attention in recent years (Kong, *et al*., 2010).

Xanthan is considered a polysaccharide synthesized by many different species of bacteria, and it forms high viscosity. Xanthan is generally soluble in cold water, is slightly resistant to enzymatic breakdown, it uses as a good cryoprotectant, and is suitable with other gums, giving better synergy with galactomannans (Dickenson, 2008).

Many researchers assessed the effects of dipping solution on the quality and shelf-life of fish products. Wangtueai *et al.,* (2014) determined the effect of a combination of NaCl and individual or mixed phosphates on some key quality parameters of frozen Nile tilapia fillets. Also, the combination effect of phosphate and modified atmosphere on quality and shelf-life extension of refrigerated seabass slices was assessed by Masniyom, *et al*., (2005), while the same effect was studied on refrigerated *Aurigequula fasciata* fillets by Ghalati *et al.*, (2017).

Therefore, this study aims to assess the effects of dipping some marine fish species (threadfin breams, goldband goatfish, marblede spinefoot and gilt-head sea bream) for 30 minutes in 5% solutions containing, sodium hexametaphosphate, sodium tripolyphosphate, chitosan and xanthan on physicochemical, microbiological and sensory quality properties of studied fish species.

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MATERIALS AND METHODS

Fish samples

Marine fish species (threadfin breams, goldband goatfish, marblede spinefoot and gilt-head sea bream) were caught during the research trip of the Yarmouk boat belonging to the National Institute of Oceanography and Fisheries during December 2020. Fish samples were carefully washed with potable water, packed in ice boxes and transported to Fish Processing and Technology Laboratory, National Institute of Oceanography and Fisheries, El-Serw Fish Research Station, Dakahlia Governorate, Egypt within three hours. Upon arrival, fish samples were rewashed with potable water, gutted, and rewashed again carefully and drained, respectively.

Fish samples treatments with dipping solutions

Sodium hexametaphosphate, sodium tripolyphosphate and xanthan were individually dissolved in the tap water to obtain final concentrations of 5 g/100 ml, while chitosan solution was prepared by dissolving 20 g of chitosan in 4973.8 ml of distilled water with 6.25 g of acetic acid, then heating with constant agitation for 24 h.

Analytical methods

Physicochemical analysis

Moisture, crude protein, fat, ash contents and pH value were carried out according to the methods recommended by the AOAC (2000). Total volatile basic nitrogen (TVB-N) and tri-methyl amine nitrogen (TMA-N) were determined according to the methods given in AMC (1979). While, the thiobarbituric acid (TBA) value was measured according to the method described by Tarladgis, *et al.* (1960).

Microbiological analysis

Ten gram of each sample were aseptically taken into a sterile homogenizer flask (Homogenizer type MPW-302, Poland), to which 90 ml of sterile (0.1%) peptone water diluted were added and homogenized for 2 minutes at 800 rpm to provide a homogenate of 10^{-1} dilution. The homogenate was thoroughly mixed. One ml of homogenate was transferred into a sterile test tube containing 9 ml of 0.1 % peptone water, then ten - fold serial dilution were prepared up to 10^{-6} Anon, (1978). Total bacterial count, total coliforms count, *Escherishia coli* and *staphylococcus aureus* were determined according to APHA (1992).

Sensory analysis

The sensory evaluation was carried out according to Mailgaad *et al.* (1999); it was done in terms of color, tenderness, taste, flavor and overall acceptability. Fish samples was evaluated by 10 panelists. A 9 point hedonic scale was employed in this sensory analysis.

Frying methods

The frying process was done in vegetable oil at 170 ºC for 1 min for all samples by using electric fryer.

Statistical analysis

All measurements were performed in triplicate and values expressed as the mean ± SD.

RESULTS AND DISCUSSION

Effect of dipping treatments on chemical composition of some marine fish species

The quality of the final food products are influenced by moisture content which has economic implications as the retention of moisture by the product increases the gross weight of the product resulting in economic gains (Sen, 2005). Effects of dipping solutions treatments on the chemical composition of some marine fish species are shown in Table (1). The obtained results showed that, the moisture content of threadfin breams, goldband goatfish, marblede spinefoot and gilt-head sea bream in control samples were 77.16, 75.13, 78.09, 76.81%, respectively, while the value of moisture content in treated fish samples ranged from77.11±0.75 to 77.90±0.54, 75.09±0.84 to 75.40±0.75, 78.11±0.55 to 78.56±0.74 and 76.20±0.89 to 76.55±0.86 %, respectively. On the other hand, the protein content of same tested fish samples were 19.68, 19.90, 18.08, 19.71%, respectively in control group, while the values of protein content (%) in treated fish samples ranged from 19.70±0.85 (SHMP treatmen) to 19.85±0.75 (chitosan treatment), 19.35±0.85 (chitosan treatment) to 19.91±0.86 (xanthan treatment), 19.25±0.86 (SHMP treatment) to19.50±0.99 (STPP treatment), 19.55±0.90 (xanthan treatment) 19.90±0.86 (xanthan treatment), respectively.

The lipid contents (%) of the same tested fish samples were 2.25, 2.45, 2.77, 2.52 %, respectively in control group, while it ranged from 2.15±0.87 to 2.25±0.92, 2.20±0.75 to 2.50±0.92, 2.70±0.70 to 2.90±0.58 and 2.45±0.82 to 2.60±0.84, respectively. The ash content (%) values of all studied fish samples (control and treated) showed that the lowest values ranged from 1.05±0.59 to1.20±0.64 in G. seabream fish and the highest values ranged from 1.70±0.89 to 1.78±0.39 in M. Spinefoot fish.

Table 1. Effect of dipping treatments on the chemical composition (%, on wet weight basis) of some marine fish species

Treatments	Fish species			
	T. breams	G. goatfish	M. Spinefoot	G. seabream
	Moisture (%)			
Control	77.16±0.59	75.13±0.44	78.09±0.78	76.81±0.77
SHMP	77.19±0.53	75.35±0.65	78.11±0.55	76.20±0.89
STPP	77.25±0.64	75.40±0.75	78.15±0.92	76.45±0.80
Chitosan	77.11±0.75	75.09±0.84	78.56±0.74	76.40±0.99
Xanthan	77.90±0.54	75.18±0.64	78.16±0.71	76.55±0.86
	Protein (%)			
Control	19.68±0.78	19.90±0.78	18.08±0.79	19.71±0.91
SHMP	19.70±0.85	19.75±0.86	19.25±0.86	19.80±0.87
STPP	19.75±0.99	19.65±0.91	19.50±0.99	19.75±0.75
Chitosan	19.85±0.75	19.35±0.85	19.28±0.75	19.90±0.86
Xanthan	19.80±0.92	19.91±0.86	19.30±0.80	19.55±0.90
	Lipids (%)			
Control	2.25±0.82	2.45±1.05	2.77±0.78	2.52±0.36
SHMP	2.20±0.55	2.30±0.90	2.80±0.66	2.55±0.65
STPP	2.15±0.87	2.20±0.75	2.90±0.58	2.50±0.99
Chitosan	2.17±0.82	2.22±0.89	2.85±0.79	2.45±0.82
Xanthan	2.25±0.92	2.50±0.92	2.70±0.70	2.60±0.84
	Ash (%)			
Control	1.38±0.53	1.65±0.98	1.78±0.45	1.08±0.98
SHMP	1.40±0.65	1.60±0.91	1.75±0.65	1.15±0.35
STPP	1.37±0.79	1.65±0.88	1.70±0.89	1.20±0.64
Chitosan	1.30±0.58	1.58±0.80	1.80±0.55	1.05±0.59
Xanthan	1.45±0.87	1.55±0.78	1.78±0.39	1.25±0.70

Each value was expressed as mean ± SD.

Effect of dipping treatments on physicochemical quality of some marine fish species

Effects of dipping solutions on physicochemical quality aspects of some marine fish species are shown in Table (2). The results showed that, the pH values of threadfin breams, goldband goatfish, marblede spinefoot and gilt-head sea bream in control samples were 6.60, 6.11, 6.19, and 6.05, respectively. The lowest pH values after treatment with dipping solutions in investigated fish species were 6.56±0.64 (STPP), 6.15±0.47 (xanthan), 6.10±0.29 (xanthan) and 6.01±0.87 (chitosan), respectively. While the highest pH values of treated fish species samples were 6.80±0.66 (xanthan), 6.18±0.77 (STPP), 6.46±0.45 (chitosan) and 6.20±0.64 (STPP), respectively.

On the other hand, the TVB-N values of same studied fish species samples were 15.52, 19.45, 17.55, 16.58 mg/100g sample, respectively in control group, while the values in treated samples ranged from 15.10±0.80 (xanthan treatment) in T. breams to 19.40±0.57 (SHMP treatment) in G. goatfish and all of these values were lower than permissible limit (30 mg TVB-N/100 g sample) recorded by connell, (1990).

TMA-N content values in control group of studied fish species samples were 0.88, 0.95, 0.78, 0.65 mg/100g sample, respectively. It was be noticed that all recorded values of TMA-N content showed a decrement after treatment with dipping solutions and the lowest value was 0.50±0.45 mg/100g (SHMP) in G. seabream fish, while the highest value was 0.85±0.11 mg/100g (xanthan) in G. goatfish. It was be concluded that all values were lower than permissible limit recoded by Connell, (1995), who demonstrated that fresh fish with less than 1-5 mg TMA-N/100g is considered as good quality while 10 -15 mg TMA-N/100g is regarded within the acceptable limits.

Table 2. Effect of dipping treatments on the physiochemical quality criteria of some marine fish species.

Treatments	Fish species			
	T. breams	**G. goatfish**	**M. Spinefoot**	**G. seabream**
	pH Value			
Control	6.60±0.75	6.11±0.35	6.19±0.54	6.05±0.46
SHMP	6.70±0.85	6.15±0.89	6.35±0.89	6.12±0.58
STPP	6.56±0.64	6.18±0.77	6.12±0.73	6.20±0.64
Chitosan	6.58±0.58	6.25±0.94	6.46±0.45	6.01±0.87
Xanthan	6.80±0.66	6.15±0.47	6.10±0.29	6.09±0.88
	TVB-N (mg/100g sample)			
Control	15.52±0.87	19.45±0.66	17.55±0.85	16.58±0.77
SHMP	15.30±0.58	19.40±0.57	17.49±0.64	16.30±0.89
STPP	15.45±0.48	19.30±0.98	17.30±0.80	16.35±0.54
Chitosan	15.18±0.83	19.20±0.74	17.28±0.94	16.40±0.56
Xanthan	15.10±0.80	19.10±0.92	17.32±0.64	16.20±0.62
	TMA-N (mg/100g sample)			
Control	0.88±0.13	0.95±0.20	0.78±0.30	0.65±0.28
SHMP	0.70±0.10	0.80±0.14	0.60±0.22	0.50±0.45
STPP	0.75±0.11	0.70±0.15	0.65±0.42	0.52±0.28
Chitosan	0.70±0.14	0.82±0.22	0.55±0.45	0.40±0.20
Xanthan	0.80±0.14	0.85±0.11	0.50±0.35	0.60±0.19
	TBA (mg MDA/1000g sample)			
Control	0.92±0.36	0.79±0.39	0.68±0.91	0.72±0.48
SHMP	0.85±0.30	0.65±0.26	0.58±0.35	0.55±0.19
STPP	0.90±0.29	0.67±0.15	0.45±0.48	0.60±0.36
Chitosan	0.80±0.09	0.55±0.52	0.66±0.09	0.50±0.91
Xanthan	0.88±0.19	0.70±0.63	0.50±0.39	0.68±0.39

Each value was expressed as mean ± SD.

According to Connell, (1990), the content values of TBA ranged from 1-2 mg MDA/kg of fish tissue are commonly considered within permissible limit. Changes in TBA values in threadfin breams, goldband goatfish, marblede spinefoot and gilt-head sea bream are presented in Table (2). The results showed that TBA content values in control group of studied fish species samples were 0.92, 0.79, 0.68, 0.72 mg MDA/kg, respectively. It was be observed that there was a decrement of TBA content values in all studied fish species samples after treatment with dipping solution and the lowest value was 0.45±0.48 mg MDA/kg (STPP) in M. Spinefoot fish, while the highest value was 0.90±0.29 mg MDA/kg (STPP) in T. breams fish. It was be concluded that all values of TBA were lower than permissible limit. These results go in parallel with those obtained by Abouel-Yazeed, (2019) who recorded that the TBA content values of control samples were higher than treated samples at zero time. Also, these results were similar to those reported by Kilinc, *et al.* (2009), which they studied the influence of dipping rainbow trout fillets for 10 min in 5% solutions containing sodium monophosphate (MSP), sodium diphosphate (DSP), and sodium triphosphate (TSP) on microbiological, chemical, color, textural, and sensory analyses of fish fillets.

Effect of dipping treatments on microbiological quality of some marine fish species

Effects of dipping solutions on microbiological quality aspects of some marine fish species are shown in Table (3). The results showed that, the total bacterial counts of threadfin breams, goldband goatfish, marblede spinefoot and gilt-head sea bream in control samples were 5.350, 5.701, 5.681and 5.255 log_{10} cfu/g sample, respectively, while it decreased in SHMP treated fish samples reaching to 5.214, 5.681, 5.587 and 5.249 log_{10} cfu/g sample, respectively. The decrement in total bacterial count was observed in all treated samples compared with control samples, and the lowest count was 5.170 log_{10} cfu/g sample (xanthan treatment) in G. seabream fish. The obtained results of TBC in all investigated fish species samples was lower than the maximam level (7 Log_{10} cfu/g of fish flesh) of microbiological criteria for fresh fish given by the ICMSF (1986), while Mohan *et al.* (2008) recommend 3×10^6 cfu/g.

Table 3. Effect of dipping treatments on the microbiological quality criteria of some marine fish species.

Treatments	Fish species			
	T. breams	**G. goatfish**	**M. Spinefoot**	**G. seabream**
	TBC (log_{10} cfu/g sample)			
Control	5.350	5.701	5.681	5.255
SHMP	5.214	5.681	5.587	5.249
STPP	5.281	5.675	5.590	5.238
Chitosan	5.264	5.668	5.559	5.235
Xanthan	5.283	5.662	5.568	5.170
	Total Coliforms count (log_{10} cfu/g sample)			
Control	4.477	3.698	3.845	3.602
SHMP	3.000	nd	3.602	3.477
STPP	3.000	3.000	3.477	3.30
Chitosan	nd	3.301	3.301	3.000
Xanthan	nd	3.477	3.301	nd

On the other hand, total coliforms count of the same studied fish samples were 4.477, 3.698, 3.845 and 3.602 log_{10} cfu/g sample, respectively in control group.

There was a decrement in total coliforms count in studied fish samples after treatment with dipping solution. It was be noticed that there were some studied fish species samples recorded as free of coliforms, while the highest coliforms count in treated samples was 3.602 (SHMP treatment) in M.

Spinefoot fish sample. Similar results were reported by Salgado, (2013) who revealed that, sardine fish treated with clove had lower colony count compared to control. Also, Kilinc, *et al*., (2009) had the same results with bacterial count in rainbow trout. The control higher than treated rainbow trout with TSP-treated samples. Wangtueai, *et al.* (2014) had a positive effect with the Nile tilapia fillets when they use of different mixed phosphates. Moreover, Marshall and Jindal (1997) reported that TSP reduced the total count of aerobic bacteria and coliform in the catfish farms and Kilinc, *et al.* (2007) has similar results with sea bass fish treated with MSP, DSP, and TSP, respectively.

Effect of dipping treatments on sensorial quality of some fried marine fish species

Effect of dipping treatments on the sensorial quality criteria of some fried marine fish species are shown in Fig. (1). The results showed that, the color values of fried threadfin breams, goldband goatfish, marblede spinefoot and gilt-head sea bream in control samples were 8.16, 8.13, 8.09, 8.11, respectively, while it increased in treated fish species samples showing the effectiveness of dipping solution on improvement color criteria. In the same trend, flavor values in control group of studied fried fish species samples were lower than treated groups. Also, it was noticed that taste, tenderness, juiciness and overall acceptability values in control group of studied fried fish spices samples were lower than treated groups.

The obtained results revealed that, sodium hexametaphosphate, sodium tripolyphosphate, chitosan and xanthan were effective for improving the sensory properties (color, flavor, taste, texture, tenderness, juiciness and overall acceptability) of the studied marine fish species samples.

The obtained results go on parallel with those reported by Ghalati, *et al.* (2017) who reported that, the addition of polyphosphate especially STPP to vacuum packed *A. fasciata* fillets has a profound effect on sensory quality, TVBN value, pH, WHC and SH and microbiological growth, and improve textural qualities. Thus, treatments with polyphosphates are an alternative way to improve the shelf life of fish.

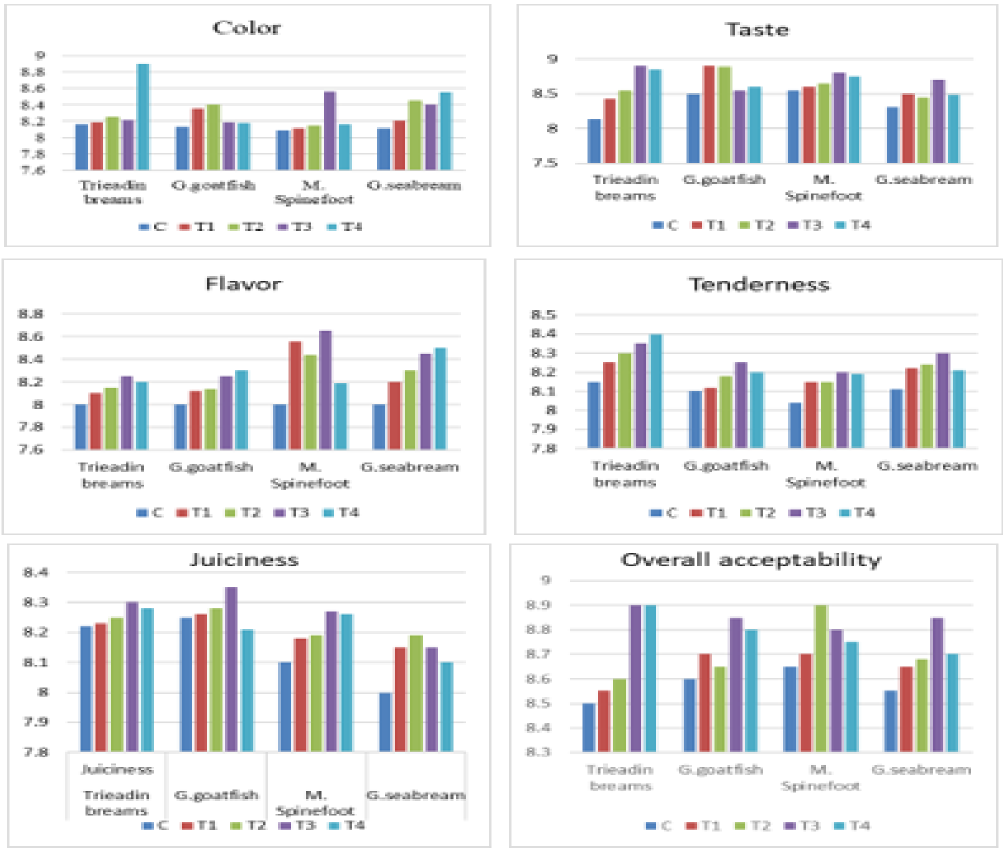

Fig. 1. Effect of dipping treatments on the sensorial quality criteria of some fried marine fish species. (C: *control*; T1: *SHMP* ; T2*: STPP* ; T3: *Chitosan* and T4: *Xanthan*.).

CONCLUSION

Treatment of threadfin breams, goldband goatfish, marblede spinefoot and gilt-head sea bream with sodium hexametaphosphate, sodium tripolyphosphate, chitosan and xanthan for 30 minutes were effective in reducing total bacterial and coliform bacterial counts. While the dipping treatments did not affect obviously on the chemical composition of studied fish species sample. On the other hand, dipping solution had an effect on physicochemical, microbiological and improving sensory properties of the tested fish samples. Referring to dipping solution, it could be concluded that, polyphosphate was the best and the most effective followed by chitosan and xanthan, so phosphate treatment can be an alternative way to improve the quality of some marine fish species.

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فاعلية بعض العوامل الواقية من التجمد على التركيب الكيميائي ومعايير الجودة الفيزيوكيميائية والميكروبيولوجية والحسية لبعض أنواع الأسماك البحرية

عبدالرحمن سعيد تعلب، مها إسماعيل جنينة و فيفى راغب أنيس

المعهد القومى لعلوم البحار والمصايد، مصر

تم إختبار تأثير بعض المواد المضادة للتجمد وهى سداسى ميتافوسفات الصوديوم ، ثلاثى فوسفات الصوديوم ، الشيتوزان والزانثان على التركيب الكيميائي وخصائص الجودة الفيزيائية والميكروبيولوجية والحسية لبعض الأسماك البحرية (الصرع، البربونى، البطاطا، الدنيس) التي تم جمعها من البحر المتوسط خلال شهر ديسمبر ٢٠٢٠ .أظهرت النتائج أن نسب الرطوبة والبروتين والدهن والرماد في أسماك الصرع، البربونى ، البطاطا، الدنيس كانت (٧٧,١٦ ، ٧٥,١٣ ، ٧٨,٠٩ ، ٧٦,٨١٪) , (١٩,٦٨ ، ١٩,٩٠ ، ١٨,٠٨ ، ١٩,٧١٪) , (٢,٢٥ ، ٢,٤٥ ، ٢,٧٧ ، ٢,٥٢٪) , (١,٣٨ ، ١,٦٥ ، ١,٧٨ ، ١,٠٨٪) على التوالي. بينما تراوحت قيم الأس الهيدروجيني، القواعد النيتروجينية الكلية المتطايرة، ثلاثى ميثيل أمين وحامض الثيوباربيتيوريك لأنواع الأسماك المختبرة من (٦,٠٥ - ٦,٦٠) ؛ (١٥,١٢ - ١٩,٣٥ ، مجم / ١٠٠ جم) ؛ (٠,٤٥ - ٠,٦٨ مجم / ١٠٠ جم) و (٠,٤٨ - ٠,٨٢ مجم مالونادهيد/ كجم) على التوالي. هذا وقد تراوحت قيم لوغاريتم العدد الكلى للبكتريا وبكتريا القولون من (٥,٢١٤ - ٥,٧٠١ و.ت.م/جم) و (٣,٠٠ - ٤,٤٧٧ و.ت.م/جم) على التوالي. هذا وكانت الخواص الحسية لعينات الأسماك المعالجة أفضل من الكنترول .والخلاصة ، أن المواد المضادة للتجمد كانت لها تأثيرات واضحة على تحسين الخصائص الفيزيائية والكيميائية والميكروبيولوجية والحسية لعينات الأسماك المختبرة بينما لم تظهر فروق في التركيب الكيميائي. من ناحية أخرى ، كان مادة عديد الفوسفات هى الأكثر فعالية يليها الكيتوزان والزانثان ، لذلك يمكن أن تكون المعالجة المسبقة بالفوسفات طريقة بديلة لتحسين جودة بعض أنواع الأسماك البحرية.