ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2022; 27(3): 3487-3497
doi: 10.25083/rbl/27.3/3487.3497

Received for publication: January, 12, 2022
Accepted: July 25, 2022

Original paper

Effect of ascorbic acid and tri-sodium phosphate treatment at different packaging conditions on cold storage Bagrus bayad fish quality

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Abstract

This research paper aimed at evaluating the influence of ascorbic acid (AA) and tri-sodium phosphate (TSP) under different packaging condition on improving the shelf life and qualities of fillets of *Bagrus bayad* cold fish at 4±1ºC for 12 days. The microbiological analysis performed were the total bacterial count (TBC), proteolytic bacterial count (PBC), lipolytic bacterial count (LBC), Coliform count (CC), *Staphylococcus aureus* (SA), *Salmonella & Shigella* (SS) and yeast & mold (YM). The obtained results indicated that all noticed analysis parameters (TBC, PBC, LBC, CC, SA, SS, YM, AA and TSP).were gradually increased during storage period in different treatments. The increases of these parameters were significantly higher ($p < 0.05$) in control sample than samples treatment with (AA) and (TSP). The obtained results also showed that there was a significant ($p<0.05$) extension of the shelf-life through delaying the microbial growth of the samples treated with (TSP) and (AA), respectively. In addition, the shelf-life and the storage period for these products were found to be less than 3 days for control sample and more than 12 days of samples treated with (TSP) under cold storage (4°C). Therefore, the (TSP) had a high effectiveness for extending the shelf-life and enhancing quality attributes of raw fish fillets during cold storage.

Keywords Bagrus bayad, ascorbic acid, tri-sodium phosphate, cold storage, quality criteria, and shelf-life

To cite this article: EL-SHENAWY MS, GOUDA MS, ELSANAT SY, OSHEBA AS. Effect of ascorbic acid and tri-sodium phosphate treatment at different packaging conditions on cold storage Bagrus bayad fish quality. *Rom Biotechnol Lett.* 2022; 27(3): 3487-3497 DOI: 10.25083/rbl/27.3/3487.3497

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Introduction

Fish is a valuable part of human nutrition because of high content of protein and polyunsaturated fatty acids with long chain (PUFAs) that are very useful due to their role in prevention of human cardiovascular (KYKKIDOU & al,[1]; ÖZOGUL & al, [2]). Fish fillets are considered better than beef and chicken meat because of their high marine protein efficiency rate (PER) and low-fat content containing long chain n-3 fatty acids, beneficial for health (CHURi & al, [3]). The expansion of the fast-food industry and the increased consumption of processed-meat products make it necessary to reevaluate the quality characteristics of fresh fish fillets products and to be available in the Egyptian market. Although fish and fish products have therapeutic and nutritional value, they have a short shelf-life, due to the vast amounts of free amino acids and volatile nitrogen bases and higher final pH that limit the useful life of the product being highly susceptible to oxidation of unsaturated fatty acids and this can affect the flavor, taste, texture, aroma, and shelf-life (MEXIS & al, [4]). The microbial activity is involved 12 million tons of seafood sector by occurring food spoilage (KULAWIK & al, [5]). To prevent and delay the quality changes that are caused by lipid oxidation in foods and seafood, various synthetic antioxidants have been used. However, with growing concerns regarding the safety of synthetic antioxidants, natural antioxidants have been utilized as a promising source of antioxidants that delay oxidative developments to preserve fish quality. This effect is referring to their favorable action to scavenge free radicals and retard the effect of pathogens through its antibacterial effects (SINGH & al, [6]). *Bagrus bayad* is so abundantly represented in the river Nile. These are generally called catfishes, of which 15 genera occur in the Nile system. Almost 36 common genus is Bagrus, which contain two species *Bagrus bayad* and *Bagrus docmac*. They reach a length of about a meter and their flesh is very good. B. bayad are extensively dispersed in river Nile and lakes. Recently, there has been increasing interest in the utilization of chemical preservatives to control microbial, oxidative and autolytic enzymatic spoilage of fish (GHALY & al, [7]). Of these, organic acids are particularly suitable as they found naturally in foods, are 'generally recognized as safe' (GRAS) (REY & al, [8]). Organic acids consider preservatives factors through lowering the pH of food and can penetrate the lipid membrane of bacteria and destabilizing of pH inside the cytoplasm causing disturbing of bacteria metabolic interaction and growth. Trisodium phosphate (TSP) as a preservative agent can reduce the bacterial growth by destructive lipid structure inside the bacterial cell membrane (MEREDIT & al, [9]; SMYTH & al, [10]). Ascorbic acid can be used as an antioxidant agent to increase shelf life of processed food and conserves. This acid is a strong antioxidant and has a direct synergistic relationship with other antioxidants (AUBOURG & al, [11]). Besides the chemical and natural preservatives agents, seafood products are requiring additional care in packaging technique to prolong their shelf life with keeping its quality. The packaging of fishery products and fresh meat inside modified atmosphere is widely exploited to delay microbial activity and enzymatic bad action (ZHANG & al,[12]) or create a satisfied protection from deteriorative effects and environmental effects. Nitrogen (N_2) is an inert, odorless, and tasteless gas. It is used as a filler to prevent package collapse, because of its low solubility in water and fat in gas packaging. The use of N_2 results in the reduction in lipid oxidation and the inhibition in the growth of aerobic spoilage microorganisms (MASNIYOM, [13]). Therefore, this study was performed to detect the effect of (AA) and tri-sodium phosphate under different packaging condition on improve the quality and shelf life of *Bagrus bayad* fish fillets during storage at 4±1ºC by determination of physico-chemical, microbial and sensory quality criteria so, shelf-life periodically during cold storage.

Materials and Methods

Materials

Whole fresh Bayad fish (*Bagrus bayad*), were obtained from river Nile near Motobus city, Kafr Elshiehk Governorate, Egypt during March, 2018. Fish samples were transported to the Laboratory of Food Technology Department, Faculty of Agriculture, Kafrelshiekh University, in insulated cooler boxes directly within 2 h of purchase and stored whole on ice in a polystyrene box in a chill room at 2ºC. The fish obtained within 48 h of landing and were of a similar weight (1500 ± 100g) and long (35± 2cm). Sunflower oil, wheat flour and NaCl obtained from local market in Kafr El-Sheikh city. Ascorbic acid and tri-sodium phosphate (Merck Co., Germany) obtained from El-Gomhoria Com. Tanta city.

Methods

Preparation of Bayad fillet

The fish samples were beheaded, gutted and washed with tap water to remove all viscera, black membranes, swim bladder and blood. Skin and bones were manually removed and aseptically filleted to produce pure fillets (162 fillet particles). The yield of flesh was about 48%. The filleted samples were exposed to cold water for quick washing and the weight of each fillet was adjusted to 200 ± 5 g. After that, three groups of samples were divided as indicated in Ta-

ble (1). The first group was considered as blank control (BC) that were submerged in sterile distilled water and directly packed in high density polyethylene (HDPE) bags. T1 submerged in 0.5% of ascorbic acid aqueous solution (AA treatment 0.5%) and T3 were submerged in 0.5% of Tri-sodium phosphate aqueous (TSP treatment 0.5%) respectively. After 5 minutes dipping time according to **(TAHERI & al, [14]),** fillets were removed from all solutions and packaged in individual HDPE bags. All samples were packaged in three different conditions: air, nitrogen and vacuum. After packaging, first the fillets were stored by refrigerated in 4°±1C for 15 days. For all types of fish fillets, microbiological analysis were carried out after 0, 6 hours and 3, 6, 9, 12 and 15 days and chemical, physico-chemical and microbial analysis were conducted in three replications.

Fish was eviscerated, de-headed, skinned & filleted. The split portions were weighed individually and their percentage of the total fish weight was calculated. The fillet percent was calculated by: Fillet % = Fillet weight × 100 Total weight.

Fish Yield: Fish was eviscerated, beheaded, skinned & filleted. The split portions were weighed individually and their percentage of the total fish weight was calculated. The fillet percent was calculated by:

$$\text{Fillet\%}=\frac{\text{Fillet weight}\times 100}{\text{Total weight}}$$

Chemical analysis

The proximate analysis of raw samples fillets was investigated according to the **AOAC, [15]).** The moisture content was determined by drying the samples in an air oven overnight at 105±2°C to a constant weight, crude protein content by using the Micro-Kjeldahl method to determine the total nitrogen and multiply its value by the factor of 6.25; ether extract, in a Soxhlet apparatus using the petroleum ether (40 -60 ºC) as a solvent; ash content by ashing in an electric muffle at 550°C until constant weight.

Determination of pH

pH values were recorded by pH meter (Jenway, 3510, UK) according to method of (GOULAS & KONTOMINAS, [16]).

Determination of total volatile basic nitrogen (TVB-N)

The (TVB-N) values were calculated by method of (KIRK & SAWYER, [17]). Briefly, the bases were steam distilled into standard acid and the back-titration was performed by standard alkali.

Determination of trimethylamine nitrogen (TMAN)

The TMAN values were calculated using the above stated TVB-N procedure after suitable modification: formaldehyde solution was utilized to slab the primary and secondary amines (**AMC, [18]).**

Determination of 2-Thiobarbituric Acid (TBA)

2-Thiobarbituric acid (TBA) value of fish samples was determined colorimetrically by using the method published by (KIRK & SAWYER, [17]). TBA values was determined as mg of malonaldehyde (MDA)/kg sample.

Water holding capacity (WHC)

(WHC) and plasticity (tenderness) were measured by following the filter press method of SOLOVIEV [19], using Placom Digetal Planimeter (KP-90 N).

Microbiological analysis

The samples of raw fish minced and fish fillets were examined for microbial profile using standard procedures **(APHA, [20])** for total bacterial count (TBC) (30°C, 3 days) and Psychrophilic bacteria (PCB) (7°C, 10 days) on plate count agar, yeast & mold (YM) counts on potato dextrose agar (21°C, 5 days). The results were expressed as log10cfu/g of sample.

Preparation of samples

Samples were prepared using the recommended methods for the microbiological examination of foods published by **(APHA, [20]).** Samples were aseptically taken using sterilized knives and placed in sterile containers. One gram of samples was emulsified in 99 ml of distilled water in a sterile mechanical blender for 1.5 min to give 1/100 dilution. Serial dilutions were prepared to be used for counting several types of bacteria and yeast and mold counts. Samples were done in triplicates.

Table 1. Fish fillets treatments.

Packaging condition	Additives (%)	Treatment
Air	Control (Untreated)	T1
Under vacuum		
Nitrogen		
Air	Ascorbic acid (AA) 0.5%	T2
Under vacuum		
Nitrogen		
Air	Tri-sodium phosphate (TSP) 0.5%	T3

A. Total bacterial counts (TBC)

TBC per one gram of sample were determined using standard techniques on plate count agar medium (Difco). Incubation was carried out at 37ºC for 48 hours, according to **APHA, [20].**

B. Coliform bacterial counts

Coliform bacterial counts were calculated by planting on crystal violet neutral red agar medium (MacConkey agar). The plates were incubated at 25ºC for 4-5 days. The colonies were counted and reported as total coliform per gram of flesh (NOTTINGHAM, [21]).

C. Proteolytic bacterial counts (PBC)

Standard-plate counts procedure were followed, 5% skim milk was added to medium; till medium became opaque. Plates were incubated for 7 days at 30 ºC. During incubation, the plates were examined at intervals for growth and clearing around the colonies. Before recording the results, the plates were flooded with 1% solution of hydrochloric acid to verify positive results. Colonies are rounded with clear zone counting proteolytic bacteria **(SALEH, [22]).**

D. Lipolytic bacterial counts (LBC)

The nutrient agar medium was used for counting the lipolytic bacterial content of the samples. Sterilized butter fat (5%) was added to every plate before pouring the medium, and vigorously hand shaking. The plates were incubated at 25°C for3 days then, the plates were flood with copper sulfate solution (20%) for10 min. Colonies appeared as blue counting lipolytic bacteria (**Manual, [23]**).

E. *Staphylococcus aureus*:

Staphylococci, which are potential pathogens capable of growing in high salt concentrations, were determined using surface planting on Baird-parker agar medium **(ICMSF, [24]).**

F. Detection of Salmonella-Shigella

The detection of *Salmonella* was performed depending on method described by (REFAI, [25]) using buffered peptone as a pre-enrichment; while tetrathionate broth was used as a selective enrichment, broth and S-S agar was used as a selective plating media.

G. Mold and yeast counts

Mold and yeast counts were determinated by plating on acidified potato dextrose agar (Difco), and incubating duplicate plates at 25°C for 5 days (NOTTINGHAM, [21]).

Organoleptic evaluation of fish

Sensory evaluation of fish was performed by twenty experienced panelists on the basis of a 10-point degree of each sample. The panelists were requested to assess the characteristics of fish treatments on a scale from 10 to 0 (GELMAN & al, [26]). The scores were given in the decreasing order scale with 10-9 for excellent, 8-7 for good, 6-5 for fair and acceptable, 4-3 for poor and 2-1 for very poor. The mean of the scores given by the panel represented the overall sensory quality. A score less than 5 indicates that the smoked herring fish is rejected.

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA). The Least Significant Difference (LSD) procedure was used to test for difference between means (significance was defined at ($p<0.05$) as reported by (SNEDECOR & COCHRAN, [27]).

Results and discussions

Proximate composition of raw Bayad fish fillets treated and untreated with ascorbic acid (AA) or tri-sodium poly phosphate (TSP):

Mean values for the chemical analysis of untreated and treated Bayad fish fillets are shown in Table (2). For raw fillets; the moisture, crude protein, fat and ash contents were 79.7, 78.1, 15.3 and 5.9% (on dry basis), respectively. From these results, it could be noticed that the investigated fish is a satisfied source of manufacturing fish fillet because belonged to high protein content and the big size of fish gave high filleting yield (48%) indicating that fish fillets that healthier than traditional meat particularly due to the presence long chain n-3 fatty acids and protein (OLIVEIRA& al, [28]). From the data shown in Table (2) it can be observed that fish fillets treated with TSP and backed with nitrogen have the best chemical properties among of the other treatments. These results are nearly accordance with obtained by (MOHAMED & al, [29]), who found that *Bagrus bayad* contains 76% moisture, 18.48% crude protein, 3.17% crude fat and 2.35% ash (on wet basis) and (MOHAMED & al, [29]) reported that the yield of mince from *Bagrus bayad* was 46.88%. Also, the obtained results are in the same line with found by (EL-SHERIF & ABD ABD EL-GHAFOUR, [30]; HUDAI, [31]). Physico-chemical changes of raw Bayad fish during storage at 4 ±1°C as affected with ascorbic acid (AA 0.5%) or tri-sodium phosphate (TSP 0.5%) at different packaging conditions on chemical composition during storage period pH value: Changes in pH value of different Bayad fish fillets during cold storage at 4 ±1°C are shown in Table (2). The differences in pH means values between different treated and untreated samples were insignificant ($P<0.05$) at zero time of storage, the initial pH values

of the control and samples treated with individually (AA) and (TSP) were 6.15 and 6.18, respectively.

Also, the results indicated a significant (*P<0.05*) increase in pH values in all fillets samples during storage period by different rates, the highest incremental rates (pH value) were detected in control sample reached to value of 6.94 at the end of storage period compared with samples treated with (AA) and (TSP) which reached to 6.30 and 6.42, respectively.

This reduction in pH values of samples treated with (AA) and (TSP) compared with control indicates the effectiveness of these (AA) and (TSP) as an antimicrobial agent so, it can be used as a way of combating the growth of common microorganisms causes of food poisoning (FISHER & PHILLIPS, [32]). The increase in pH values of all samples treated with (0.5%) (AA) and (TSP) and untreated during the storage period due to the protolytic enzymes that hydrolyzed fish protein to simple proteins, polypeptides and amino acids which were nutritious intermediate compounds as reported by (KHALLAF, [33]). The obtained results were in the same trend of those reported by (ÖZPOLAT & al, [34]). Total volatile basic-nitrogen (TVB-N) content: The impact of the individual tested control, ascorbic acid (AA) and tri-sodium poly phosphate (TSP) on TVB-N values of cold stored fish fillets was showed in Table (2), there were significant differences (*p<0.05*) in TVB-N values between treated fish fillets samples (AA) and (TSP) and the control (untreated) at the beginning of storage.

It is clear from the present results that at the beginning of the storage, TVB-N value was 11.60 mg/100g (on wet basis) for control fillets samples while, were 10.0 and 8.3 mg/100g for samples treated with (AA) and tri-sodium poly phosphate, respectively. During cold storage, the significant (p=0.05) increasing pattern in TVB-N values was observed in all investigated fish fillets samples. This may be due to the breakdown of proteins resulted from proteolytic enzymes and microbial activity (EL-SHERIF & ABD EL-GHAFOUR [30]; KHALLAF, [33]). The highest significant (*p<0.05*) incremental of TVB-N value was recorded in control sample. The treatments with (AA) and (TSP) respectively, were more active in deferring the rate of TVB-N increase during the cold storage period.

This may be due to the role of such chemical agents on microbial population and bacterial growth as antimicrobial agents (SACCHETTI & al, [35]). At the end of cold storage period, the TVB-N values for all samples except control did not exceed the acceptable limit stipulated and mentioned by **EOS, [36]** they stated that 20 mg TVB-N/100g raw samples indicates the spoilage of fish and minced meat.

Thus, control sample of investigated fish fillets considered spoiled (22.15mg/100g) at 15 days of storage while, (AA) and (TSP) sample (15.45 mg/100g) were not surpass the favorable limit at the end of storage period (15 days). The formation of TVB-N is generally related with the activity of micro-organisms and the formation tends to be increase at high microbial population (Chytiri & al, [37]). This finding proves the effect of (AA) and (TSP) in reduction of the bacterial growth then, TVB-N. From results; (AA) and (TSP) were the high effective of Bayad fish fillets in retarding the rate of TVB-N increase, as well as in prolonging the shelf-life of fish fillets throughout the subsequent cold storage (at 4 ± 1ºC for 15 days) and treatments with (AA) and (TSP) could be arranged descending according to the reduced levels of TVB-N as follows: fish fillets treated with (AA) and (TSP) respectively. These results are in agreement with reported by (EL-SHERIF & ABD EL-GHAFOUR, [30]; OSHEBA & al, [38]; ÖZPOLAT, & al, [34]). Trimethylamine Nitrogen (TMAN): is responsible for the pleasant fishy odor that produced from partly effect of intrinsic enzymes and through the bacterial activity **(SHAKILA& al, [39]).**

Changes in TMAN of different bayad fillets treatments during cold storage are shown in Table 2. From statistical analysis of these data it could be noticed that, their significant differences (*p>0.05*) were recorded in TMAN values between all treatments immediately after processing.

Meanwhile, TMAN values of all other treatments not exceeded this limit at any time of cold storage. From Table 2, it could be noticed that, TMNA values were significant decreased in TSP treatment especially with nitrogen compared with both control sample and other treatments. TMAN of treatment prepared with TSP reached the value 9.2 mg N/100 g after12 days of cold storage, exceeding the upper acceptability limit set by **(EOS, [36])** for TMNA values of fish products (10 mg N/100 g).

Thiobarbeturic acid (TBA) value

The influence of individually (AA) and (TSP) treatments on the TBA values of Bayad fish fillets during cold storage at 4±1ºC for 15 days was determined. It could be noticed from Table 2 that immediately after preparation (at zero time of storage) there were no significant (*p<0.05*) variances between the control and other treated samples. The highest value of TBA was found in untreated fish fillets sample; control (0.45 mg malonaldehyde (MDA)/kg flesh), while the lowest value was noted in samples treated with (AA) (0.26 mg MDA/kg) followed by samples treated with (TSP) (0.38 mg MDA). These values of TBA were increased (*p<0.05*) to 1.85 and 2.05 mg MDA/kg flesh, respectively at the end of storage period (15 days). Therefore, although the (AA) and (TSP) had significant (*p<0.05*) reduction effect on the TBA values, there was an increasing in

TBA values in all different fish fillets samples throughout the cold storage by different rates affecting by (AA) and (TSP) and cold storage period.

The incremental in TBA values for all fish fillets samples with increasing the cold storage time may be due to the self-oxidation of fats, bacteriological and/or oxidative rancidity. Thus, the control sample considered spoiled at 5 days of storage (2.15 mg MDA) and sample treated with (AA) (2.05 mg MDA) spoiled at 15 days as reported by (SUMAN & al, [40]) who indicate that TBA values of 2 mg MDA/kg flesh or greater in meat such as beef are considered to be rancid and (BONNELL, [41]) mentioned that fish and fish products of good quality will have TBA value less than 2mg MDA/kg flesh, while poorer quality fish will have 3-27mg

Table 2. Effect of treatment Bayad (*Bagrus bayad)* with ascorbic acid (AA 0.5%) or tri-sodium phosphate (TSP 0.5%) at different packaging conditions on chemical composition during storage period (on dry weight basis).

Storage period (Day)				Sale time (hour)		Packaging condition	Treatment	Chemical parameters
12	9	6	3	6	0			
--	--	76.5	77.2	78.0	79.7	Air^{c}		
--	--	77.0	77.5	78.6	79.7	Vacuum^{b}	Control (Untreated)^{B}	
--	--	78.0	78.4	79.3	80.0	Nitrogen^{a}		
--	75.7	76.4	77	77.5	79.1	Air^{c}		
--	76.0	76.7	77.3	78.0	79.3	Vacuum^{b}	Ascorbic acid (0.5 %)^{C}	Moisture (%)
--	76.4	77.2	78.0	79.2	79.9	Nitrogen^{a}		
--	76.2	77.0	78.0	79.2	80.0	Air^{b}		
--	77.4	78.2	79.0	79.5	80.1	Vacuum^{b}	Tri-sodium phosphate (0.5 %)^{A}	
--	78.1	79.3	80.0	80.6	80.1	Nitrogen^{a}		
--	--	76.7	77.2	77.6	78.1	Air^{c}		
--	--	77.2	77.4	77.9	78.2	Vacuum^{b}	Control (Untreated)^{A}	
--	--	77.3	77.7	78.2	78.4	Nitrogen^{a}		
76.5	77.0	77.4	77.7	78.0	78.2	Air^{b}		
76.8	77.2	77.5	77.9	78.1	78.3	Vacuum^{ab}	Ascorbic acid (0.5 %)^{AB}	Crude protein (%)
77.0	77.4	77.6	77.8	78.0	78.2	Nitrogen^{a}		
76.7	77.1	77.3	77.6	77.8	78.0	Air^{c}		
76.9	77.3	77.5	77.7	77.9	78.1	Vacuum^{b}	Tri-sodium phosphate (0.5 %)^{B}	
77.0	77.3	77.7	78.0	78.2	78.4	Nitrogen^{a}		
--	--	14.7	15.0	15.2	15.3	Air^{c}		
--	--	14.9	15.1	15.2	15.3	Vacuum^{b}	Control (Untreated)^{A}	
--	--	15.0	15.2	15.3	15.4	Nitrogen^{a}		
14.7	14.9	15.1	15.2	15.3	15.4	Air^{c}		
14.8	15.0	15.1	15.2	15.3	15.4	Vacuum^{b}	Ascorbic acid (0.5 %)^{A}	Ether extract (%)
14.6	15.0	15.1	15.2	15.3	15.4	Nitrogen^{a}		
14.6	14.8	15.0	15.2	15.3	15.4	Air^{c}		
14.7	14.9	15.1	15.2	15.3	15.4	Vacuum^{b}	Tri-sodium phosphate (0.5 %)^{B}	
14.8	15.1	15.2	15.2	15.3	15.4	Nitrogen^{a}		
--	--	7.5	6.8	6.4	5.9	Air^{a}		
--	--	7.4	6.8	6.2	5.9	Vacuum^{ab}	Control (Untreated)^{C}	
--	--	7.3	6.7	6.3	5.9	Nitrogen^{b}		
7.8	7.6	7.2	6.7	6.3	5.9	Air^{a}		
7.7	7.3	7.0	6.6	6.3	5.9	Vacuum^{ab}	Ascorbic acid (0.5 %)^{B}	Ash content (%)
7.5	7.1	6.7	6.3	6.1	5.7	Nitrogen^{b}		
7.9	7.5	7.3	6.8	6.4	6.0	Air^{a}		
7.8	7.4	7.2	6.8	6.3	6.0	Vacuum^{b}	Tri-sodium phosphate (0.5 %)^{B}	
7.8	7.4	7.2	6.7	6.3	6.0	Nitrogen^{b}		

All values given are means of three determinations.

Values followed by different letter in row are significantly different at $p<0.05$.

(a.b and c) : comparison of means by packaging condition at ($P<0.05$) by Duncan's multiple comparison tests.

A, B and C: Comparison of means by treatment at ($P<0.05$) by Duncan's multiple comparison tests.

MDA/kg. Accordingly, the fillets samples treated with ginger or rosemary in the current study would not deceive consumers up to 15 days of storage. Finally, the obtained results revealed that the treatment of Bayad fish fillets with (AA) and (TSP), respectively led to extension ($p<0.05$) of their shelf-life comparing with the control sample. These results are in agreement with previous studies by (EL-SHERIF & ABD EL-GHAFOUR, [30]; UÇAK & al, [42]).

Physical changes of raw Bayad fish during storage at 4 ±1°C as affected by (AA) or tri-sodium poly phosphate

As shown in Table (3), water holding and Tenderness values were significantly decreased with increasing storage period. Table (3): Effect of treatment Bayad (*Bagrus bayad*) with ascorbic acid (AA) or tri-sodium phosphate (TSP) on Physical parameters during storage period.

Microbiological load changes of raw Bayad fish fillets during storage at 4 ±1 °C as affected by (AA) or tri-sodium poly phosphate

Total bacterial count (TBC)

Table (4) shows the changes of total bacterial count of different fish fillets samples during cold storage as affected by treated by (AA) and (TSP) individually. At zero time of storage, control samples (untreated) recorded the highest mean counts of TBC comparing to other samples treated with (AA) and (TSP), that caused a significantly ($p<0.05$) reduction in microbial count of treated fish fillets immediately after preparation. The TBC of control and treatment samples with (AA) and (TSP) fish fillets were gradually increased significantly ($p<0.05$) during storage at 4±1ºC with a highly increase in control and depending on the kind treatment. The growth of psychrophilic bacteria under this storage condition might cause the noticed increase (OSHEBA & al, [38]). TBC (cfu/g flesh) of control sample were 3.25 at zero time of storage increased to 7.65 cfu/g fillets sample at the end of storage period (15 days), while samples treated with (AA) and (TSP) were 2.60 and 3.02 (cfu/g flesh) at zero time increased to 3.70 and 4.00 (cfu g^{-1}), respectively. This mentioned that (AA) and (TSP) may be responsible for the antibacterial properties. Also, it could be detected that significant differences ($P<0.05$) (TSP) was noticed have strong inhibitory effect against the growth of TBC followed by (AA). In this study the raw fish fillets of control sample was considered accepted until 6th day of storage and were rejected because TBC count was exceeded than the maximum permissible level MPL (10^6 numbers/g) was reported the acceptable limit of TBC for fil-

Table 3. Effect of treatment Bayad (*Bagrus bayad)* with ascorbic acid (AA) or tri-sodium phosphate (TSP) on Physical parameters during storage period.

Storage period (Day)				Sale time (hour)		Packaging condition	Treatment	Physical parameters
12	9	6	3	6	0			
--	--	4.80	4.6	4.5	4.41	Aira	Control (Untreated)A	Water holding capacity (WHC) (g water retained /g protein)
--	--	4.71	4.6	4.53	4.40	Vacuuma		
--	--	4.63	4.55	4.46	4.38	Nitrogenb		
4.79	4.7	4.63	4.45	4.4	4.35	Aira	Ascorbic acid (0.5 %)A	
4.75	4.63	4.60	4.45	4.39	4.33	Vacuumab		
4.72	4.68	4.55	4.43	4.37	4.32	Nitrogenb		
4.57	4.45	4.37	4.3	4.20	4.11	Aira	Tri-sodium phosphate (0.5 %)B	
4.50	4.38	4.28	4.17	4.10	4.00	Vacuumab		
4.44	4.30	4.19	4.11	4.05	3.95	Nitrogenb		
--	--	3.00	3.10	3.15	3.24	Aira	ControlA (Untreated)	Tenderness
--	--	3.30	3.12	3.17	3.20	Vacuuma		
--	--	3.00	3.05	3.12	3.17	Nitrogenb		
2.77	2.83	2.92	3.00	3.08	3.15	Aira	Ascorbic acid (0.5 %)B	
2.65	2.77	2.85	2.96	3.04	3.12	Vacuumb		
2.61	2.75	2.83	2.94	3.02	3.08	Nitrogenb		
257	2.65	2.73	2.85	2.94	3.00	Aira	Tri-sodium phosphate (0.5 %)C	
2.54	2.63	2.70	2.82	2.90	2.97	Vacuumb		
2.50	2.60	2.67	2.75	2.80	2.94	Nitrogenc		

All values given are means of three determinations.
Values followed by different letter in row are significantly different at p<0.05.
(a.b and c) : comparison of means by packaging condition at ($P<0.05$) by Duncan's multiple comparison tests.
A, B and C: Comparison of means by treatment at ($P<0.05$).

lets, also MPL recommended by the International Commission on Microbiological Specifications for TBC in all fillets products is below 7 log10cfu/g sample **(ICMSF, [24]),** while the other fish fillets samples treated with (AA) and (TSP) remained acceptable microbiologically and safe for consumption up to 15 days storage at 4±1ºC because the values were below the MPL. Therefore, this study demonstrated that the use of (0.5%) (TSP) decreased the microbial attendance of cold raw Bayad fish fillets compared to the control. These results are in agreement with found by (ÖZPOLAT & al, [34]) and (BABATUNDE & ADEWUMI, [43])

Yeast & Mold (YM) counts

Results in Table (4) show the changes in the yeast and mold of the different types of Bayad fish fillets during refrigerated storage at 4±1ºC affected by addition individually (AA) and (TSP), at preparation. YM counts were not detected in any of fillets samples at the 0th day until 6thday of storage. YM counts were detected in control samples (1.85 cfu/g) at 5th day. In treated fillets samples with (TSP), it could be noticed that YM counts were appeared at the 10th day in samples treated with (AA) (1.22 cfu/g) and at 15th day in samples treated with (TSP) (1.20 cfu/g). On the other

Table 4. Effect of treatment Bayad (*Bagrus bayad)* with ascorbic acid (AA) or tri-sodium phosphate (TSP) on total viable bacterial, molds & yeast, psychrophilic bacterial, Lipoletic bacteria and Protoletic bacteria counts during storage period.

Storage period (Day)			Sale time (hour)			Packaging condition	Treatment	Microbiological parameters
12	9	6	3	6	0			
-	-	7.2	4.8	3.2	1.8	Air^{a}		
-	-	6.5	3.6	2.5	1.6	Vacuum^{b}	Control (Untreated)^{B}	
-	-	5.9	3.4	2.3	1.5	Nitrogen^{c}		
7.8	6.0	5.2	3.4	2.7	1.7	Air^{a}		Total bacterial counts (CFU/g ×10^{5})
6.9	5.3	4.1	2.8	2.4	1.5	Vacuum^{b}	Ascorbic acid (0.5 %)^{A}	
5.8	4.9	3.8	2.5	2.0	1.5	Nitrogen^{c}		
7.1	5.7	4.5	3.1	2.3	1.6	Air^{a}		
6.4	4.7	3.4	2.9	2.1	1.4	Vacuum^{b}	Tri-sodium phosphate (0.5 %)^{C}	
5.6	4.4	3.2	2.5	1.8	1.4	Nitrogen^{c}		
-	-	7.4	5.7	3.8	2.2	Air^{a}		
-	-	7.0	5.3	3.5	2.0	Vacuum^{b}	Control (Untreated)^{A}	
-	-	6.8	4.7	3.3	2.0	Nitrogen^{c}		
7.4	6.3	5.5	4.1	3.4	2.0	Air^{a}		Molds & yeast counts (CFU/g ×10^{3})
6.9	5.4	4.6	3.7	2.7	1.9	Vacuum^{b}	Ascorbic acid^{C} (0.5 %)	
6.6	4.9	4.1	3.3	2.5	1.8	Nitrogen^{c}		
8.4	6.7	5.9	4.4	3.6	2.1	Air^{a}		
8.1	6.2	5.3	3.9	3.2	1.9	Vacuum^{b}	Tri-sodium phosphate^{B} (0.5 %)	
7.5	5.9	4.4	3.4	2.9	1.9	Nitrogen^{c}		
-	-	7.0	5.3	4.1	3.0	Air^{a}		
-	-	6.6	5.0	3.9	2.8	Vacuum^{b}	Control (Untreated)^{A}	
-	-	6.0	4.5	3.8	2.6	Nitrogen^{c}		
8.1	6.3	4.9	3.8	3.0	2.5	Air^{a}		Lipoletic bacteria count (CFU/g ×10^{2})
7.2	6.8	4.2	3.5	2.9	2.3	Vacuum^{b}	Ascorbic acid (0.5 %)^{B}	
6.7	5.5	4.0	3.5	2.7	2.1	Nitrogen^{a}		
7.5	5.8	4.6	3.7	3.0	2.4	Air^{a}		
6.6	5.4	4.3	3.4	2.7	2.2	Vacuum^{b}	Tri-sodium phosphate (0.5 %)^{A}	
6.3	5.0	4.1	3.2	2.5	2.0	Nitrogen^{c}		
		5.7	4.3	3.1	2.3	Air^{a}		
		5.2	4.0	2.7	1.8	Vacuum^{b}	Control (Untreated)^{C}	
		4.8	3.7	2.5	1.8	Nitrogen^{c}		
7.2	5.6	4.8	3.4	2.6	1.8	Air^{c}		Protoletic bacteria count (CFU/g×10^{3})
6.7	5.1	4.4	3.3	2.4	1.7	Vacuum^{a}	Ascorbic acid (0.5 %)^{B}	
6.4	4.9	4.0	3.2	2.2	1.6	Nitrogen^{b}		
7.0	5.5	4.5	3.3	2.4	1.6	Air^{a}		
6.3	5.0	4.2	3.1	2.3	1.6	Vacuum^{b}	Tri-sodium phosphate (0.5 %)^{C}	
6.0	4.8	4.0	2.8	2.0	1.6	Nitrogen^{c}		

All values given are means of three determinations.

Values followed by different letter in row are significantly different at $p<0.05$.

(a.b and c) : comparison of means by packaging condition at ($P<0.05$) by Duncan's multiple comparison tests.

A, B and C: Comparison of means by treatment at ($P<0.05$).

Table 5. Organoleptic properties of fried Bayad (*Bagrus bayad)* treated with ascorbic acid (AA) or tri-sodium phosphate (TSP) at zero time

Treatment	Organoleptic properties score					
	Appearance	Color	Odor	Texture	Taste	Overall acceptability
ControlC (Untreated)	8.5	7.5	8.3	8.0	8.1	8.08
Ascorbic acidB (0.5%)	8.5	8.2	8.7	8.5	7.8	8.34
Tri-sodium phosphateA (0.5%)	9.0	8.7	8.6	8.6	8.8	8.74
Significance (P <0.05)						

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.
a, b, c and d: Comparison of means by salting time.

hand, YM counts were gradually increased for all fish fillets samples during cold storage as noticed in Table (4) and a highly significant differences (*P<0.05*) between the control sample and other samples treated with (AA) and (TSP) were noticed thus, the control sample was highest (*p<0.05*) count until the final of storage period. At the end of storage period, YM counts reached to (4.00 cfu/g) of control fillets samples, while 2.55 and 2.90 of samples treated with (AA) and (TSP), respectively. Therefore, the fillets sample treated with (TSP) was the lowest YM counts followed by samples treated with (AA) compared with control sample, this indicated that (AA) and (TSP) have stronger effects against the growth of yeast and molds than control in reduction of yeast and molds population. So, the additions of (AA) and (TSP) to fish fillets were extended product shelf life (15 day) compared to the control (15 day). These results are in agreement with reported by (EL-SHERIF & ABD EL-GHAFOUR, [30]; IHEAGWARA, [44]; ÖZPOLAT & al, [34]). Finally, all cold storage fish fillets treatments were completely free from colifrom bacteria, *staphylococcus aureus*, *salmonella* and *Shigella* either at a zero-time or along cold-storage periods, indicating good level of hygiene during the handling, processing and storage.

Sensory properties changes of Bayad fish fillets during cold storage at (4±1ºC) as affected by (AA) and (TSP):

The results of sensory analysis are one of the most important quality criteria used for determination of shelf life of seafood. The changes of sensory properties (taste, color, odor, texture and general acceptability) of fried Bayad fish fillets treated individually with (AA) and (TSP) and untreated (control) during cold storage at (4±1ºC) were recorded in Table (5). It could be found that Bayad fish fillets at zero time of storage had excellent scores for color and texture then very good scores for flavor (taste and odor) immediately after preparation. Therefore, a noticeable significant (*p<0.05*) difference could be observed between samples treated with EOs and control in the case of taste and odor characteristics during the storage period. **EOS, [36]** was found to have an added advantage in terms of their synergistic effect against oxidation and in enhancing the taste and odor. The decline in overall acceptability of control samples as a result of this difference in taste and odor was more prominent during 15-20 days of storage and the off flavor formation was occurred. While, there was no marked difference (*p<0.05*) between the EOs treated samples and control in color and texture during zero time till 10 days of storage then, a significant (*p<0.05*) difference was found until the end of storage period. On the other hand, the taste, color, odor, texture and overall acceptability scores of control sample reached to 4.0, 4.5, 4.5, 4.2 and 4.2, respectively by 20 days of storage which was below the acceptable limit of score (5) and was greatly deteriorated being undesired as softening of texture and off odors was appeared then, this product become rejected for consumers.

In the case of the other samples treated with EOs, the scores were above of acceptable limit even after the end of storage period (25 days). Hence, the scores of overall acceptability garlic, ginger and rosemary samples were 5.3, 6.5 and 5.8 respectively at the end of storage period (25 days) and was in the order samples, ginger > rosemary > garlic. Therefore, Bayad fish fillets samples treated with 1.0% ginger showed the significant (p<0.05) highest mean score of overall acceptability followed by samples treated with 1.0% rosemary and treated with 1.0% garlic compared with untreated samples (control) which rejected before 20 days of cold storage.

Even through the added EOs delayed oxidation and extending the shelf-life, their antioxidant activity reduced during storage by increase significantly (*p<0.05*) differences in all individual tested samples during the end of storage periods.

Conclusion

In conclusion, the results obtained in this study showed the tested ascorbic acid and tri-sodium phosphate had / or presented / antimicrobial properties that improved the physico-chemical, microbiological and sensory quality attributes of raw Bayad fish fillets during cold storage thus, extending the

shelf life. This is justified by the low TVB-N, TBA, and pH as well as bacteria, yeast and mold counts of fish fillets related with EOS than untreated control samples. Sensory evaluation revealed that shelf life of fish fillets samples was longest ($P < 0.05$) for samples treated with TSP as compared to the control (12 days) under storage at4±1ºC. Therefore, the present findings recommended that the TSP and AA should be utilized for extending the shelf-life and enhancing quality attributes and sensory properties of fish fillets during cold storage.

Acknowledgments

The study was supported by a project funded by department of Food Science and Technology, Kafrelshiekh University.

Conflict of interest

The authors have declared that there is no conflict of interest.

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