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INFLUENCE OF SODIUM ALGINATE WITH ASCORBIC ACID COATING ON THE QUALITY OF CATFISH (*CLARIAS GARIEPINUS*) FILLETS DURING COLD STORAGE

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Abstract

The effect of alginate coating containing ascorbic acid on shelf-life and the quality of catfish (*Clarias gariepinus*) fillets was evaluated during cold storage. Catfish fillets were left untreated as control (C) or treated with 5% ascorbic acid (T1), sodium alginate coating 1.5 % (T2) or coating with sodium alginate 1.5 % containing ascorbic acid 5% (T3). All fillet samples were immersed in the solutions for 10 min. Then the T2 and T3 immersed in 2 % (w/v) calcium chloride to gel for 2 min. The samples were stored at (4 ±1ºC) for 15 days and determined the pH as well as analyzed for chemical (protein, fat, total volatile basic nitrogen, trimethylamine nitrogen and thiobarbituric acid values), microbiological (total bacterial counts, yeasts and molds counts) and sensory properties (odor, texture, appearance and taste).

The results indicated that all the treatments retarded the decay of the catfish fillets as compared to untreated fillets (C). Coating treatments predominantly reduced chemical spoilage, reflected in pH, total volatile base nitrogen, trimethylamine, and thiobarbituric acid values as compared to those of T1 treated fillets. T3 followed by T2 then T1 treatment showed more efficient inhibition in the growth of total bacteria, yeasts and molds and increased the overall sensory scores of fish fillets compared to untreated fillets during stored at (4 ±1ºC) for 15 days.

Keywords: Sodium alginate. Ascorbic acid. Catfish fillets. Cold storage.

INTRODUCTION

After fish is harvested, its storage period is limited. Though low temperature can delay the rate of fish deterioration and also extend the shelf life to some extent, the quality of fish muscle will still deteriorate during cold storage. Microbial activity and enzymes contained in fish tissues also degrade

the muscle protein resulting in the quality loss of fish. Deterioration of fish muscle mostly occurs in the fat-containing portions. Fatty acids are affected by the environmental oxygen that oxidizes and spoils the fish meat (Kilincceker *et al*., 2009). So taking some measures to delay the decline of fish quality and extend the preservation life of fish, through inhibiting, or retarding the growth of microorganisms and reducing the rate of lipid oxidation is necessary.

Coating the chilled foods with edible materials has been researched as an effective method to inhibit quality loss. Hydrophilic edible films are good barrier for oxygen and carbon dioxide and possess suitable mechanical properties at low relative humidity. Also, edible films offer alternative packaging systems, which may replace some synthetic packaging materials or reduce their application by partial replacement (Haque *et al*., 2009).

Alignates are polysaccharides extracted from anionic red or brown seaweed; *Phaeophycase* and also from giant kelp; *Macrocystis pyrifera* (Song *et al*., 2011). They are linear polymer of D-mannuronic acid and linear polymer of D-mannuronic acid and L- gulucouronic acid. It is used as sodium or calcium salt in the food system (Khan *et al*., 2013). Alginate has unique colloidal properties and can form strong gels or insoluble polymers through cross-linking with post treatment by $CaCl_2$ solution. Such biopolymer-based films can keep good quality and prolong shelf life of foods by preventing microbe contamination, maintaining the flavor, reducing fat oxidation and weight loss (Lu *et al*., 2009). Moreover, the coatings may serve as carriers for antimicrobial compounds and antioxidant in order to maintain high concentrations of preservatives on the surface of foods. A few antimicrobial agents and antioxidants have been incorporated into edible coatings to suppress quality changes during storage (Chidanandaiah *et al*., 2009).

Ascorbic acid (Vitamin C) is well known as a natural antioxidant. It demonstrates potential for its use as anti-oxidant in food industry especially in the field of meat manufacture. Ascorbic acid plays an important role in enzyme

inhibition, reduce oxygen and carbon centered radicals as well as chelated metal ions (Gregory, 1996).

Nowadays, African catfish (*Clarias gariepinus*) are considered as valuable species not only in Egypt, but also in many other parts of the world because of their high growth rates, tolerance of a wide range of temperature and dissolved oxygen levels (Amisah *et al*., 2009). It is an important source of cheap and high-quality protein. In addition, catfish is a lean and highly nutritious fish that is rich in vitamins, minerals and low in carbohydrates as well as have a good palatability (Ersoy and Yılmaz, 2003). This study was conducted to further investigate the efficacy of sodium alginate solution containing ascorbic acid as edible coating on shelf-life and the quality of catfish (*Clarias gariepinus*) fillets during storage at 4 ±1ºC for 15 days.

MATERIALS AND METHODS

Samples preparation and treatments:

30 Kg catfish (*Clarias gariepinus*) (1600 - 1570 g for each) was obtained from Central Laboratory for Aquaculture Research, Egypt. Catfish transferred directly to the laboratory alive, immediately the head and all fins were hand removed using a sharp knife. The whole fish was eviscerated, filleted and washed carefully in water.

Sodium alginate (Food-grade sodium alginate, pH 7·0, Sigma Company, Saint Louis, MO, USA) was used for the coating formulations. Sodium alginate solution 1.5% was prepared by mixing 15 g of sodium alginate with 1000 ml of distilled water. The mixture was heated on a hot plate at temperature of 80 ºC with constant stirring until completely dissolved and become clear. Ascorbic acid 5% was prepared (El-Nasr Pharmaceutical and Chemical Company, Egypt). Then the solution was made up to 1000 ml with distilled water. Calcium chloride 2% (w/v) (pH 7·0, $CaCl_2$, El-Nasr Pharmaceutical and Chemical Company, Egypt) used to induce the cross-linking reaction also was prepared. All solutions were cooled to room temperature prior to surface application onto fillet samples.

Catfish fillets samples were divided into four equal treatments as follows: Untreated control (C) dipped in distilled water; (T1) dipped in 5% ascorbic acid; (T2) dipped in sodium alginate solution 1.5% and (T3) dipped in mixture of sodium alginate 1.5% and ascorbic acid 5% solution. Catfish fillet samples were immersed in the solutions for 10 min. After dipping, fillets were allowed to drain for 5 min on a sterile stainless wire mesh screen at 25°C±1ºC, air-dried for 2 min. and then T2 and T3 fillet samples immersed in calcium chloride 2% (w/v) to gel formation for 2 min. They were then packed in polyethylene bags, tied off and stored at 4±1^{o}C for 15 days. Samples were taken randomly every 3 days intervals for analysis. All the analyses were made in three replicates.

Physico-chemical analysis:

The total protein and fat contents were determined according to the methods described in AOAC (2005). The values of pH was estimated according to Özogul *et al.* (2005) method, using pH-meter (Orion Research Digital Ion analyzer, Model 420 a). Thiobarbituric acid TBA values mg malondialdehyde /kg (mg MDA/kg fish flesh) were determined as described by Kirk and Sawyer (1991). Colorimetric absorbance at 530 nm was measured using a Spectronic 710 Spectrophotometer. Readings were converted to TBA mg MDA/kg fish flesh. Total volatile bases nitrogen values (TVB-N) mgN/100g fish flesh were determined as described by Kirk and Sawyer (1991) while trimethylamine nitrogen values (TMA-N) mg N/100 fish flesh were determined according to AMC (1979).

Microbiological analysis:

Total bacterial counts (TBC) were counted on plate count agar following incubation at 30°C for 3 days as recommended in (AOAC, 2005). Total yeasts and molds counts (TYMC) were enumerated on Oxytetracycline glucose yeast extracts agar (Oxoid CM 545) after incubation at 22°C for 3 - 5 days as described by the APHA (1992). Colonies were counted and reported as logarithms of the number of colony-forming units ($\log_{10}$ cfu/g).

Organoleptic evaluation:

Panelists were asked to evaluate treated raw catfish fillet samples for appearance, texture and odor. The cooked samples assessed for taste. Before presented to the panelists, fish fillet samples were cooked in a microwave oven (a Litton Menu-master system 70/50) operating at 2450 MHZ) for 10 minutes at medium temperature. The cooked samples were served hot to panelists Özoğul *et al.* (2011). A group of 10 judges were always called upon for scoring beginning grads ranging from zero to 10 as ascribed by Teeny and Miyauchi (1972) according to the following scheme:

Score	Description	Score	Description
10	Ideal	4	Fair
9	Excellent	3	Poorly fair
8	Very good	2	Poor
7	Good	1	Very poor
6	Fairly good	0	Repulsive
5	Acceptable		

Statistical analysis:

The results were analyzed using One-way analysis of variance (ANOVA) and means comparison was performed by Duncan's multiple range tests (Steel and Torrie, 1980). Statistical analysis was carried out using SPSS statistic program (Version 14.0) for Windows Evaluation Version (SPSS Inc. Chicago, IL).

RESULTS AND DISCUSSION

Chemical composition:

The results in Table (1) indicated that the initial protein and fat contents of catfish fillets samples were 71.95 and 12.26%, respectively. According to Connell (1995) the initial levels of protein and fat may be attributed to the

chemical composition of fish itself which closely related to fish nutrition, fish species and catching season.

At the end of storage period the protein content was 66.42, 68.93, 69.34 and 70.92% and fat content reached to 8.82, 10.15, 10.42 and 11.21 % in control (C), T1, T2 and T3 samples, respectively. Regarding to theese results there was a significant decrease in protein and fat content during storage period in (C) samples with storage time ($P < 0.05$) compared to T1, T2 and T3 treated fillets. The levels of protein and fat were C< T1< T2< T3 treatment. Similar results were obtained by Chomnawang *et al.* (2007) they revealed that the decrease in total protein content of fish flesh is due to the decrease in water soluble protein and salt soluble protein. Gandotra *et al.* (2012) showed significant decrease in protein and lipid in the muscle of *Mystus seenghala* during storage at 4±1°C. They attributed the decrease in fat and protein contents to the protein denaturation, hydrolysis and fat oxidation.

Table 1. Effect of sodium alginate and ascorbic acid coating on total protein and lipids contents of catfish fillets during storage at 4±1°C (on dry weight basis)*.

Parameter		Total protein (%)*				Total lipids (%)*			
Treatments		C	T1	T2	T3	C	T1	T2	T3
Storage period (days)	0	71.95± 0.03	71.95± 0.02	71.95± 0.01	71.95± 0.01	12.26± 0.02^{a}	12.26± 0.02^{a}	12.26± 0.02^{a}	12.26± 0.03^{a}
	3	71.32± 0.05^{ab}	71.42± 0.05^{a}	71.60± 0.04^{a}	71.73± 0.05^{a}	11.71± 0.05^{ab}	12.14± 0.05^{b}	12.12± 0.04^{b}	12.21± 0.06^{a}
	6	70.70± 0.06^{b}	70.90± 0.05^{ab}	71.30± 0.05^{a}	71.61± 0.06^{a}	11.03± 0.03^{c}	12.84± 0.04^{ab}	11.89± 0.04^{b}	11.98± 0.03^{a}
	9	70.01± 0.04^{bc}	70.40± 0.05^{b}	70.94± 0.04^{ab}	71.47± 0.04^{a}	10.35± 0.03^{c}	11.23± 0.06^{ab}	11.41± 0.04^{b}	11.75± 0.05^{a}
	12	69.10± 0.03^{bc}	69.89± 0.04^{b}	70.61± 0.04^{ab}	71.29± 0.03^{a}	9.74± 0.02^{d}	10.82± 0.05^{ab}	11.02± 0.04^{b}	11.54± 0.03^{a}
	15	66.42± 0.04^{d}	68.93± 0.05^{c}	69.34± 0.04^{b}	70.92± 0.03^{a}	8.82± 0.02^{d}	10.15± 0.03^{c}	10.42± 0.04^{b}	11.21± 0.04^{a}

$^{a-d}$ Means within a raw with the same superscript significantly different ($P<0.05$).

Values are expressed as Mean ± SD. C: Untreated control.

T1: Ascorbic acid 5%; T2: Sodium alginate 1.5%.

T3: Mixture of sodium alginate 1.5% and ascorbic acid 5%.

On the other hand, Oriakpono and Ndome (2012) mentioned that, the loss of this protein and lipid content was due to activities of endogenous enzymes followed by the growth of microorganisms. The highest levels of protein and fat in T3 treatment may be attributed to the synergic effects of coating by sodium alginate and ascorbic acid. Similar results were obtained by Khan *et al.* (2013).

Physico-chemical evaluation:

The pH values of fillet samples are shown in Table (2). The initial pH values were 6.65, 6.43, 6.46 and 6.37 for control C, T1, T2 and T3 samples, respectively. The values of the pH at zero day for fillets treated with sodium alginate and /or ascorbic acid (T1, T2 and T3) might be attributed to the effect of treatments on samples. The change in pH of the samples showed that the values slightly decreased initially from zero day to 3rd day and then gradual significant increased ($P<0.05$). At the end of storage the pH values were 7.38; 6.85; 6.79 and 6.54 for C, T1, T2 and T3 samples, respectively. According to Fan, *et al*, (2009) the initial decrease of pH value may be due to the attack of rigor mortis which led to decomposition of glycogen and formation of lactic acid and/or protein denaturation. Also, formation of amino and free fatty acids which were produced during the storage period. In this respect Erkan and Ozden (2008) stated that the increase of pH value may be due to increase of volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes. Our results are in an agreement with Wang *et al.* (2003) they found that the pH value of Atlantic salmon muscle increased from 6.32 to 6.80 after storage for 14 days at 4ºC. Gandotra *et al.* (2012) observed that the pH increased from 6.8 to 7.4 in muscle of *Mystus seenghala* during 21 days at 4±1ºC. Also, Oriakpono and Ndome (2012) founded that the pH of *Tilapia Guineensis* was increased from 6.81 to 7.20 during storage at 4ºC for 4 weeks. However, it could be mentioned that the reduction in pH values T3< T2< T1 for the treated samples may be attributed to calcium alginate coating, and /or ascorbic acid might inhibit microbial growth, decrease the activity of the endogenous proteases and retard protein breakdown. This trend is in accordance with Fan *et al.* (2009) and Song *et al.* (2011).

The value of thiobarbituric acid (TBA) mg MDA/kg is an index of lipid oxidation. According to Goulas and Kontominas (2007), TBA value of 2 (mg MDA/kg) of fish flesh is usually regarded as the limit beyond which fish will normally develop an objectionable odor and taste.

Table 2. Effect of sodium alginate and ascorbic acid coating on pH and thiobarbituric acid (TBA) values (mg MDA/kg) of catfish fillets during storage at 4±1ºC (on wet weight bases).

Parameter		pH				TBA (mg MDA/kg)			
Treatments		C	T1	T2	T3	C	T1	T2	T3
Storage period (days)	0	6.65± 0.06^{a}	6.43± 0.05^{a}	6.46± 0.02^{a}	6.38± 0.05^{b}	0.25 ± 0.003^{a}	0.25 ± 0.003^{a}	0.25 ± 0.001^{a}	0.25 ± 0.003^{a}
	3	6.61± 0.05^{a}	6.40± 0.005^{b}	6.43± 0.03^{b}	6.34± 0.02^{c}	1.56± 0.01^{a}	0.91± 0.01^{b}	0.83± 0.02^{ab}	0.55± 0.007^{c}
	6	6.76± 0.005^{a}	6.48± 0.017^{b}	6.47± 0.005^{b}	6.31± 0.09^{c}	2.02± 0.02^{a}	1.22± 0.01^{b}	1.18± 0.02^{ab}	0.86± 0.01^{c}
	9	6.84± 0.01^{a}	6.57± 0.01^{b}	6.51± 0.02^{b}	6.35± 0.05^{c}	3.61± 0.03^{a}	1.93± 0.02^{b}	1.81± 0.02^{c}	1.37± 0.01^{d}
	12	7.19± 0.006^{a}	6.66± 0.01^{b}	6.64± 0.01^{c}	6.49± 0.07^{d}	4.27± 0.05^{a}	2.86± 0.03^{b}	2.51± 0.04^{c}	1.76± 0.02^{d}
	15	7.38± 0.21^{a}	6.85± 0.006^{b}	6.79± 0.006^{c}	6.54± 0.08^{d}	6.51± 0.08^{a}	3.78± 0.04^{b}	3.69± 0.06^{c}	2.06± 0.02^{d}

$^{a-d}$ Means within a raw with the same superscript significantly different (P<0.05).

Values are expressed as Mean ± SD. C: Untreated control.

T1: Ascorbic acid 5%; T2: Sodium alginate 1.5%.

T3: Mixture of sodium alginate 1.5% and ascorbic acid 5%.

The results showed that the initial TBA value was 0.25 (mg MDA/kg fish flesh) for all catfish samples (Table 2). The TBA values of all samples increased continuously during storage. The values of TBA reached to 3.61 and 2.86 (mg MDA/kg fish flesh) on day 9 and 12 for (C) and (T1) fillet samples, respectively, while TBA values were 2.51 and 2.06 (mg MDA/kg fish flesh) on day 12 and day 15 for (T2) and (T3) treated fillet samples, respectively. The values of TBA increased may be attributed to the oxidation of unsaturated fatty acids in fish fillets by various mechanisms including enzymatic and non-enzymatic, generation of free radicals and active oxygen (Aubourg, 2001). However, the treated samples T3, T2 and T1 were significantly ($p < 0.05$) lower than that of (C) samples throughout the storage period at 4±1ºC. The treatment (T3) had the best effect due to synergistic effects of ascorbic acid with the

alginate-based film on fillets surface which may have been resistant to oxygen diffusion, thus retarded lipid oxidation (Song *et al.*, 2011). Similar observations were reported by Zeng and Xu (1997) and Kilincceker *et al*. (2009) which they coated fish, shrimps and scallops with sodium alginate and found that it could control lipid oxidation effectively. According to Ozer and Sariçoban (2010) the antioxidant mechanism of ascorbic acid is due to its oxygen scavenger agent and a metal chelation activity. Similar findings were reported by Chidanandaiah *et al.* (2009) and Lu *et al.* (2009).

The Total volatile basic nitrogen (TVB-N) value is a usual indicator of fish flesh protein deterioration (Kyrana *et al*. 1997). On the other hand, the trimethylamineoxide (TMAO) is a part of the non-protein-nitrogen (NPN) fraction. A number of well defined spoilage bacteria are able to utilize TMAO to trimethylamine nitrogen (TMA-N) (Gram and Huss, 1996). Table (3) shows the effects of different treatments on TVB-N and TMA-N production for the catfish fillet samples stored at 4±1ºC. The initial TVB-N and TMA-N values were 8.93 and 2.14 mg N/100g, respectively for all samples. In this connection, Kirk and Sawyer (1991) reported that, at level of 20 mg N/100g for TVB-N in fish muscle is usually regarded as fresh. Levels of 30– 35 mg TVBN/100 g in muscle are generally considered to be the acceptable limit for cold-water fish stored on ice (Connell, 1995). While the acceptable European Union (EU) limits of the TMA-N content, 10-15 mg/100 g (Connell, 1995).

The TVB-N and TMA-N values of all samples showed a slow increase in the early storage, but a marked significant increase ($P < 0.05$) in TVB-N and TMA-N were observed after 3 days for control and treated samples. The increase in TVB-N and TMA-N during storage may be attributed to the activity of spoilage bacteria and endogenous enzymes (Lu *et al*., 2009).

After 15 days of storage the values of TVB-N and TMA-N followed the order: T3 < T2 < T1< C samples. The values revealed that, the application of ascorbic acid and sodium alginate coating (T3 fillet samples) was more effective as compared to T1 and T2 treatments for extending the shelf life and decreasing protein deterioration. The current results are in agreement with Aubourg (2001) in Horse mackerel, Song *et al*. (2011) in bream (*Megalobrama amblycephala*) and Chidanandaiah *et al.* (2009) in buffalo meat cuts. Song *et al*.

(2011) reported that fish samples treated by ascorbic acid led to either rapidly reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds. The sodium alginate coating without an antioxidant could extend the shelf life of catfish fillets by reducing water loss. These results are in agreement with that of Lu *et al.* (2009) who observed that alginate calcium coating slowed the development of TVB-N relative to untreated northern snake head fillets.

Table 3. Effect of sodium alginate and ascorbic acid coating on the total volatile bases nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) mg N/100g values of catfish fillets during storage at 4±1ºC (on wet weight bases).

Parameter		Total volatile bases nitrogen (mg N/100g)				Trimethylamine nitrogen (mg N/100g)			
Treatments		C	T1	T2	T3	C	T1	T2	T3
Storage period (days)	0	8.93± 0.06^{a}	8.93± 0.06^{a}	8.93± 0.06^{a}	8.93± 0.07^{a}	2.14± 0.03^{a}	2.14± 0.03^{a}	2.14± 0.04^{a}	2.14± 0.03^{a}
	3	16.00± 0.02^{a}	14.40± 0.01^{b}	13.14± 0.02^{c}	10.40± 0.01^{d}	6.690± 0.05^{a}	4.19± 0.04^{b}	3.18± 0.04^{c}	2.33± 0.03^{d}
	6	23.90± 0.03^{a}	20.85± 0.05^{b}	18.20± 0.03^{c}	12.91± 0.04^{d}	14.82± 0.04^{a}	9.11± 0.05^{b}	8.90± 0.05^{c}	4.89± 0.04^{d}
	9	36.58± 0.03^{a}	27.50± 0.04^{b}	26.80± 0.04^{c}	18.23± 0.05^{d}	17.21± 0.07^{a}	14.17± 0.05^{b}	13.27± 0.05^{c}	6.78± 0.05^{d}
	12	43.17± 0.04^{a}	36.12± 0.005^{b}	35.93± 0.06^{c}	23.89± 0.05^{d}	19.96± 0.1^{a}	16.95± 0.8^{b}	16.57± 0.06^{c}	9.77± 0.05^{d}
	15	50.78± 0.005^{a}	44.52± 0.005^{b}	43.91± 0.06^{c}	30.32± 0.05^{d}	22.60± 0.2^{a}	19.30± 0.1^{b}	18.21± 0.08^{c}	12.17± 0.07^{d}

$^{a-d}$ Means within a raw with the same superscript significantly different ($P<0.05$).

Values are expressed as Mean ± SD. C: Untreated control.

T1: Ascorbic acid 5%; T2: Sodium alginate 1.5%.

T3: Mixture of sodium alginate 1.5% and ascorbic acid 5%.

Microbiological evaluation:

Variations in the values of the total bacterial counts (TBC) in the catfish fillets during storage are presented in Table (4). The initial numbers of bacteria in the fillet samples were 3.59, 3.41, 3.44 and 3.11 $\log_{10}$ cfu/g for control (C), (T1), (T2) and (T3) samples, respectively.

The dipping of fillets in the different solutions resulted in variations in the reduction of the initial bacterial contents. Chytiri *et al.* (2004) observed that initial TBC of filleted rainbow trout was 3.8 $\log_{10}$ cfu/g and reported that, the initial bacterial counts of different freshwater fish species ranged between 2.00 and 6.00 $\log_{10}$ cfu/g. The initial microbial load of freshwater fish varies depending on water conditions and temperature. The results also revealed that, from day 0 to day 3 of the storage period, there was minor increased in the TBC for treated fillet samples, in contrast to the extreme significant increase ($p>0.05$) in control (C) samples.

From day 3 the TBC of all samples increased by increasing storage time. There were significant differences ($p > 0.05$) between C, T1 and T2, T3 fillet samples, which indicates that T1, T2 and T3 strongly inhibited the growth of TBC but T3 had the best effect as compared to untreated control and other treatments. Meanwhile, the TBC of (C) fillet samples reached to 9.75 $\log_{10}$ cfu/g on day 9, which exceeded the maximum acceptable count of 10^7 cfu/g for freshwater as recommended by ICMSF (2011), while T1 and T2, treatments reached to 7.52 and 7.23 $\log_{10}$ cfu/g on day 15, respectively. Regarding, the TBC of T3 samples it did not exceed the limit value (5.24 $\log_{10}$ cfu/g) during storage at 4±1ºC for 15 days. T3 treatment more efficiently inhibited the growth of bacteria ($p > 0.05$) followed by T2 then T1 samples. The results were in accordance with those reported by Fang and Tsai (2003); Chytiri *et al.* (2004) and Lu *et al*., (2009).

Table (4) represented total yeasts and molds counts (TYMC) of catfish fillets. It could be noticed that the initial TYMC of control (C) was 1.53 $\log_{10}$ cfu/g, while treated samples T1, T2 and T3 counted 1.15, 1.17 and 1.12 $\log_{10}$ cfu/g, respectively. The increment of TYMC of untreated control reached to 6.96 ($\log_{10}$ cfu/g) after 9 days. The control samples (C) were completely rejected after 6 days because the visual appearance of mold spots on its surface. While TYMC reached to 3.41 and 3.21 ($\log_{10}$ cfu/g) for T1 and T2 samples after 12 day. T1 and T2 samples were completely rejected by the panelists because of the visual appearance of mold spots on its surface after 12 day of

storage period. Elevated TBC and TYMC of T1 and T2 treated samples at the end of cold storage period may be due to pH, water activity, and composition of the food (Song *et al*., 2011).

Table 4. Effect of sodium alginate and ascorbic acid coating on the total bacterial count (TBC) and total yeasts and molds count (TYMC) of catfish fillets during storage at 4±1ºC.

Parameter		TBC (log_{10} CFU/g)				TYMC (log_{10} CFU/g)			
Treatments		C	T1	T2	T3	C	T1	T2	T3
Storage period (days)	0	3.59± 0.03^{a}	3.41± 0.02^{a}	3.44± 0.03^{a}	3.11± 0.02^{a}	1.53± 0.04^{a}	1.15± 0.05^{a}	1.17± 0.04^{a}	1.12± 0.05^{a}
	3	5.47± 0.04^{a}	3.91± 0.03^{ab}	3.85± 0.04^{ab}	3.16± 0.03^{b}	2.29± 0.08^{a}	1.29± 0.04^{b}	1.39± 0.06^{ab}	1.18± 0.04^{b}
	6	6.38± 0.05^{a}	4.82± 0.04^{bc}	4.62± 0.05^{b}	4.30± 0.03^{c}	3.01± 0.06^{a}	1.81± 0.04^{b}	1.41± 0.01^{c}	1.19± 0.06^{ab}
	9	9.75± 0.06^{a}	5.41± 0.05^{bc}	5.21± 0.05^{b}	4.55± 0.04^{c}	6.96± 0.05^{a}	2.21± 0.02^{b}	2.11± 0.03^{c}	1.29± 0.06^{d}
	12	Spoiled	6.84 ± 0.06^{a}	6.64± 0.06^{b}	4.87± 0.05^{c}	Spoiled	3.41± 0.04^{a}	3.21± 0.08^{b}	1.31± 0.02^{c}
	15	Spoiled	7.52± 0.05^{a}	7.23± 0.03^{b}	5.24± 0.04^{c}	Spoiled	4.82± 0.01^{a}	4.51± 0.02^{b}	1.43± 0.03^{c}

$^{a-d}$ Means within a raw with the same superscript significantly different (P<0.05).
Values are expressed as Mean ± SD. C: Untreated control.
T1: Ascorbic acid 5%; T2: Sodium alginate 1.5%.
T3: Mixture of sodium alginate 1.5% and ascorbic acid 5%.

The total yeasts and molds counts (TYMC) for T3 were significantly (P < 0.05) lower followed by T2 then T1 treatments as compared to the control samples. The lower TBC and TYMC in T3 fillets samples may be due to effects of the alginate coating as a barrier against oxygen transfer with ascorbates leading to inhibition of growth of the aerobic bacteria as well as molds and yeasts. This result matches with those reported by Chidanandaiah *et al.* (2009) they proved that, the alginate coatings with ascorbates lower the microbial growth because of its antifungal effect, meanwhile ascorbates may have the same antimicrobial mechanism by chelating the metal ions required for microbial growth. Also, the results agree with Fang and Tsai (2003) they found that alginate – calcium gels in combination with antimicrobial agents (chelators as organic acids) was more efficiently suppress the microbial growth in ground beef than did alginate–calcium gels or antimicrobial agents alone.

Organoleptic evaluation:

Tables (5) and (6) shows the effect of sodium alginate and ascorbic acid coating on appearance, texture, odor and taste scores for catfish fillets during storage at 4±1ºC. All sensory scores were significantly ($P<0.05$) declined for control (C), (T1), (T2) and (T3) samples during storage period. The sensory scores order of catfish fillets samples were T3> T1 >T2 > C. It is obvious that (C) fillets had the lowest scores and completely rejected by the panelists after 6 days of cold storage due to their petrifaction, flabby texture, bad color and taste. Whereas, the sensory acceptability limit was 9 days for treated (T1) and (T2). According to Suvanich *et al*., (2000); Olsson *et al.* (2003) and Manat *et al.* (2005) the decrease in sensory scores attributed to autolysis and spoilage bacteria which were able to utilize the protein. Lipolysis and fat oxidation are the major factors of changes in the organoleptic properties during storage period. These results are in agreement with those documented by Olsson *et al.* (2003); Chytiri *et al.*, 2004 and Oriakpono and Ndome (2012).

On the other hand, the taste scores of T2 treated catfish fillets with sodium alginate only may be attributed to the $CaCl_2$ treatment, which could affect the fillets by producing a faintly bitter taste. Also (Williams *et al.,* 2004) revealed that, free calcium used for fixing alginate coatings may increase proteolytic enzyme activity on meat surfaces by acting as enzyme activators which may decrease sensory scores. These results are in agreement with those reported by Lu *et al.* (2009) and Khan *et al*. (2013).

The T3 treatment fillets accepted up to 12 days may be due to inactivation of microorganisms and enzymes in fillets by coating with sodium alginate containing ascorbic acid. These results are in line with those obtained by Chidanandaiah *et al.* (2009) they mentioned that alginate coatings improved the texture and color of meat. Song *et al*., (2011) reported that, ascorbic acid was used in fish flesh to maintain the color and prevent the off-flavor.

Finally this study revealed that catfish fillets treated with ascorbic acid incorporated in alginate coating efficiently inhibited the growth of microorganisms, reduced the degree of chemical spoilage, improved antioxidant activity and enhanced the overall sensory values leading to extending the shelf life during storage for 15 days at 4±1ºC than that untreated control fillets and individual treatment with alginate or ascorbate.

Table 5. Effect of sodium alginate and ascorbic acid coating on appearance and texture of catfish fillets during storage at 4±1ºC.

Parameter		Appearance				Texture			
Treatments		C	T1	T2	T3	C	T1	T2	T3
Storage period (days)	0	8.90± 0.071^{a}	8.90± 0.072^{a}	8.90± 0.072^{a}	8.90± 0.073^{a}	9.00± 0.05^{a}	9.00± 0.06^{a}	9.00± 0.05^{a}	9.00± 0.05^{a}
	3	7.80± 0.066^{c}	8.10± 0.070^{b}	8.05± 0.073^{b}	8.60± 0.074^{a}	7.00± 0.07^{b}	8.20± 0.04^{ab}	8.50± 0.04^{a}	8.80± 0.05^{a}
	6	5.60± 0.054^{d}	7. 32± 0.063^{c}	7.28± 0.071^{b}	8.30± 0.072^{a}	5.70± 0.06^{b}	7.50± 0.05^{ab}	7.80± 0.04^{a}	7.90± 0.04^{a}
	9	3.60± 0.03^{d}	5.40± 0.056^{c}	6.50± 0.064^{b}	7.80± 0.071^{a}	3.80± 0.05^{b}	5.50± 0.05^{ab}	6.70± 0.06^{a}	7.00± 0.07^{a}
	12	2.50±® 0.04^{d}	3.70± 0.03^{c}	3.90± 0.04^{b}	6.20± 0.08^{a}	2.20±® 0.02^{d}	3.60± 0.06^{b}	3.80± 0.05^{ab}	6.10± 0.06^{a}
	15	1.50±® 0.03^{d}	2.80±® 0.001^{c}	3.00±® 0.03^{b}	5.10± 0.05^{a}	1.40±® 0.04^{d}	2.70±® 0.002^{c}	3.01±® 0.003^{c}	5.10± 0.06^{a}

$^{a-d}$ Means within a raw with the same superscript significantly different (P<0.05).
Values are expressed as Mean ± SD. C: Untreated control.
T1: Ascorbic acid 5%; T2: Sodium alginate 1.5%.
T3: Mixture of sodium alginate 1.5% and ascorbic acid 5%. ®: Rejected.

Table 6. Effect of sodium alginate and ascorbic acid coating on the odor and taste of catfish fillets during storage at 4±1ºC.

Parameter		Odor				Taste			
Treatments		C	T1	T2	T3	C	T1	T2	T3
Storage period (days)	0	9.10± 0.09^{a}	9.10± 0.08^{a}	9.10± 0.08^{a}	9.10± 0.09^{a}	9.00± 0.05^{a}	9.00± 0.05^{a}	9.00± 0.06^{a}	9.00± 0.05^{a}
	3	7.00± 0.07^{c}	8.50± 0.08^{b}	8.60± 0.08^{a}	8.80± 0.08^{b}	7.00± 0.07^{d}	8.57± 0.04^{c}	7.76± 0.04^{b}	8.70± 0.05^{a}
	6	5.80± 0.05^{c}	7.20± 0.06^{b}	7.40± 0.07^{a}	8.30± 0.08^{a}	5.30± 0.06^{d}	7.39± 0.04^{c}	6.60± 0.05^{b}	7.90± 0.04^{a}
	9	3.41± 0.04^{c}	5.70± 0.04^{b}	6.11± 0.05^{a}	7.20± 0.06^{a}	3.00± 0.05^{d}	5.40± 0.06^{c}	5.20± 0.05^{b}	7.00± 0.07^{a}
	12	2.40±® 0.03^{d}	3.40± 0.04^{c}	3.90± 0.04^{b}	6.60± 0.05^{a}	2.50±® 0.04^{d}	3.50± 0.04^{c}	3.30± 0.03^{c}	6.10± 0.06^{a}

15	1.70±® 0.02^{d}	2.30±® 0.01^{c}	2.40±® 0.01^{b}	4.90± 0.05^{a}	1.80±® 0.02^{d}	2.20±® 0.01^{c}	2.10±® 0.001^{b}	4.80± 0.04^{a}

$^{a-d}$ Means within a raw with the same superscript significantly different (P<0.05).

Values are expressed as Mean ± SD. C: Untreated control.

T1: Ascorbic acid 5%; T2: Sodium alginate 1.5%.

T3: Mixture of sodium alginate 1.5% and ascorbic acid 5%. ®: Rejected.

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تأثيرالتغليف بألجينات الصوديوم وحمض الأسكوربيك على جودة شرائح سمك القراميط أثناء التخزين بالتبريد

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تم دراسة تأثير التغليف بألجينات الصوديوم وحمض الاسكوربيك على جودة وصلاحية شرائح سمك القراميط أثناء التخزين بالتبريد (٤ ± ١) °م لمدة ١٥ يوم. تم تقسيم شرائح سمك القراميط الى ٤ معاملات غمرت لمدة عشرة دقائق فى محاليل على النحو التالي: مجموعة الكنترول من شرائح سمك القراميط (C) غمرت في ماء مقطر، معاملة T1 {غمرت في حمض الأسكوربيك (5٪)}، معاملة T2 {غمرت بألجينات الصوديوم (١.٥٪)}، ومعاملة T3 {غمرت في مخلوط ألجينات الصوديوم(١.٥٪) مع حمض الأسكوربيك (5٪). شرائح سمك القراميط T3 وT2 تم غمرها بعد ذلك مدة دقيقتان فى كلوريد الكالسيوم (٢٪). تم قياس الأس الهيدروجيني (pH) و اجراء الاختبارات الكيميائيه (بروتين، دهن، المركبات القاعدية النيتروجينية الكلية الطيارة ، والنيتروجين ثلاثي ميثيل أمين ، قيم حمض الثيوباربتيوريك) والميكروبيولوجيه (العدد الكلي للبكتريا والفطريات والخمائر) وكذلك الخصائص الحسية (الرائحة، القوام، المظهر والطعم).

أوضحت النتائج أن جميع المعاملات تؤخر فساد شرائح القراميط مقارنة مع شرائح الكنترول. كما أشارت النتائج إلى أن شرائح سمك القراميط التى غمرت فى محلول ألجينات الصوديوم خفضت الفساد الكيميائي المبين في قيم (pH ، المركبات القاعدية النيتروجينية الكلية الطيارة والنيتروجين ثلاثي ميثيل أمين ،حمض الثيوباربتيوريك) مقارنة مع شرائح T1 المعاملة بحمض الأسكوربيك فقط. كما تبين ان T3المعاملة بمخلوط ألجينات الصوديوم (١.٥٪) مع حمض الأسكوربيك (5٪) أكثر فعالية تليها T2 ثمT1 في تثبيط نمو البكتيريا والخمائر والفطريات مع زيادة الجودة الحسية لشرائح سمك القراميط مقارنة بالكنترول أثناء التخزين بالتبريد (٤ ± ١) °م لمدة ١٥ يوم.