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EFFECT OF SOME COATING SOLUTIONS ON THE QUALITY OF VACUUM-PACKED FISH FILLETS

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ABSTRACT

This study was carried out to examine the effect of dip treatment of different coatings of lactic acid, acetic acid, acetic + lactic, chitosan, lactic + chitosan or acetic + chitosan on the shelf life of vacuum-packed lout fish (*Sciaena umbra*) during chill storage assessed by chemical, microbiological and sensory parameters. The results revealed that the coated fillets presented a remarkable and significant reduction in thiobarbituric acid number "TBA" values compared to control samples during chill storage. In all coating treatments, the TBA values were found to be within the safe limit (35-40 mg) throughout the storage period. Trimethylamine "TMA" in the tested samples showed significant differences between all groups during storage period especially between the coated and uncoated control fillets. In this study, only the uncoated control samples reached spoilage limit after 16 days of storage; meanwhile all other coated samples were within the acceptable limit throughout the whole storage period. Moreover, it was obvious to notice that the most effective treatment was chitosan + acetic followed by chitosan + lactic and then chitosan. The use of chitosan +acetic and vacuum-packaging was found to be very efficient in extending the shelf life of fish fillets samples in the sensory point of view. Thus, vacuum-packaging in conjunction with a chitosan + acetic coating can be used safely to extend the shelf life of fish fillets up to 28 days at 4°C (±1).

Keywords: Fish fillet, freshness, Vacuum packaging, Chitosan, invisible films, storage time.

INTRODUCTION

Fish are a rich source of high-quality proteins, essential vitamins and healthful polyunsaturated fatty acids. Fish are perhaps one of the most vulnerable of the world's resources. For many economically developing nations, fish are the first or second largest export commodity. Since the freshness of fish deteriorates rapidly, freshness may be considered a synonym for quality. The increasing demand for high quality fresh seafood has intensified the search for methods and technologies for better fresh fish utilization. One of the major developments in food packaging is packaging under vacuum or modified atmosphere conditions. Vacuum-packaging represents a static form of hypobaric storage. It is widely used in the food industry because of its effectiveness in reducing oxidative reactions in the product at relatively low cost. This method of packaging can be a supplement to ice or refrigeration to delay spoilage, extend the shelf life, maintain a high quality, assure the safety and reduce economic loss of fish and fishery products. (Ioannis.*et al.,* 2005).

Fish and shellfish are highly perishable, because of their high water activity a_w, neutral pH, and presence of autolytic enzymes. The rate of

deterioration is highly temperature dependent and can be inhibited by the use of low storage temperature (e.g. fish stored on ice). The spoilage of fresh fish is usually microbial. However, in some cases chemical changes, such as auto oxidation or enzymatic hydrolysis of the lipid fraction may result in off-odors and off-flavors as well as tissue enzyme activity can lead to unacceptable softening of the fish (Huss *et al*., 1997). In microbiological point of view, fish muscle is sterile at the time of catching, but becomes quickly contaminated by surface and intestinal bacteria and from equipment and humans during handling and processing. During chilled storage, there is a shift in bacterial types. Psychrotrophs, *Pseudomonas* and *Shewanella* dominate the microflora after 1-2 weeks of storage. At higher temperatures (25°C), the microflora at the point of spoilage is dominated by mesophilic *Vibrionaceae* and *Enterobacteriaceae* particularly if the fish are caught in polluted waters (Huss, 1995). However, microbial spoilage of food products is not adequately controlled, and preventing contamination with pathogens is occasionally unsuccessful. Modified atmosphere packaging (MAP) has acquired special importance for fish products, because it results in a significant increase of shelf-life as a consequence of the inhibitive effect of CO_2 on aerobic gram-negative flora (Pastoriza *et al*., 2002). Modified atmosphere packaging (MAP) in combination with refrigeration has proven to be an effective preservation method for the extension of shelf-life of fresh fish and fish products (Sivertsvik *et al*., 2002).

Due to the perishability of such a product, reliable methods of preservation are sought to extend shelf life and to avoid health hazards. Such method includes cold storage, low-dose, cook-chill process and treatment with organic acids and their salts such as lactic acid and acetic acid which can inhibit many pathogens microorganisms (Kim *et al*, 1995). Chitosan-based materials may be used as edible films or coatings due to their unique property of increased viscosity upon hydration. Furthermore, chitosan films are tough, long-lasting, flexible, and very difficult to tear. Most mechanical properties of chitosan films are comparable to those of many medium-strength commercial polymers (Butler, 1996).

The main aim of this work was to study the effect of vacuum-packaging with and without dip treatment of different coatings of lactic acid, acetic acid, acetic +lactic, chitosan, Chitosan +lactic or chitosan +acetic on the shelf life of lout fish (*Sciaena umbra*) during chill storage assessed by chemical, microbiological and sensory parameters.

MATERIALS AND METHODS

1. Fillets preparation and coating treatments

Thirty kilograms of lout fishes (*Sciaena umbra*) of superior quality were purchased from a retail fish store, Ismailia Governorate, Egypt. The purchased fish were of the same size with average weight varied from 2 to 2.5 kg per one fish. Fish were transferred immediately to the laboratory in a cool box for fillet preparation and analysis. Each fish was cleaned from any dirt and then the fish head was cut and the scales were entirely removed. The cleaned fish was gutted, filleted and then cut into 15×5cm steaks. Fillet

steaks were randomly divided into seven groups. One group was kept without coating treatment to serve as a control. The fillets of the other six groups were treated with only one coat by immersing fillets of each group in one of the following solutions for 3 min: (Chitosan 1%, Acetic acid 1%, Lactic acid 1%, Acetic 1%+lactic 1%, Chitosan 1%+Acetic 1%, Chitosan 1%+lactic 1%). 6Fillets were then picked up carefully from coating solutions and then allowed to dry in air for tow hours at room temperature (20±1°C). Dried fillets with formed invisible edible films were vacuum packed using a vacuum-packaging machine (Decosonic Inc, Model KIC 828, China). Coated, vacuum-packed fillet samples were stored at 4±1°C in a refrigerator. Chemical analysis (pH, titratable acidity, weight loss, total volatile basic nitrogen "TVBN", trimethylamine "TMA" and thiobarbituric acid number "TBA") and microbial analysis (Total bacterial counts, yeast and molds, psychrotrophs, *Pseudomonas* spp., and *Aeromonas* spp.) were carried out at 3 days intervals to determine the overall quality of fish fillets during storage.

1.1. Preparation of chitosan coating solution

For preparing 1% chitosan-coating solutions, 10g chitosan (Sigma-Aldrich, Oakville, Canada) was added to 1.0 liter deionized water containing 10g of glacial acetic acid. The mixture was stirred at 40 °C for 1h using a magnetic stirrer/hotplate. Glycerol was added to the solution as a plasticizer at a level of 1.0 ml/g chitosan and stirred for additional 10 min. The resultant chitosan coating solution was filtered to remove any undissolved particles.

2. Chemical analysis

2.1. Titratable and acidity pH

Values of pH were determined according to Apha (1998) using a pH meter (Jenway 3305, U.K.), and the titratable acidity was expressed as g lactic acid per 100 g fresh weight.

2.2. Determination of TVB-N, TMA and TBA.

TVB-N, TMA and TBA values were determined according to Kirk and Sawyer. (1991). The amount of TVB-N or TMA was expresses as mg/100g. The amount of TBA was expressed as mg malonaldehyde per kg sample.

3. Microbiological enumeration

For bacterial enumeration, 10 g of each fillet sample was homogenized in 90 ml 0.1% peptone water using a homogenizer (Universal laboratory AID type MPW, Japan). Then, serial dilutions up to 10^6 were prepared from the original dilution. Dilutions were spread-plated in duplicate, on plat count agar (Difco, 1984) for enumeration of psychrotrophic aerobic bacteria. *Pseudomonas* bacteria were counted by *Pseudomonas* isolation agar (Oxoid) according to Difco (1984). *Aeromonas* bacteria were determined by Starch Ampicillin Agar (SAA) according to Harmon *et al*. (1992). Plates were incubated for 10 days at 7˚ C for psychrotrophic aerobic bacteria, and for 2 days at 28˚C for *Pseudomonas* and *Aeromonas*.

4. Sensory evaluation

Sensory characteristics of samples were evaluated by an eight-member panel according to the method recommended by Nielsen (1995) as shown in Table 1.

5. Statistical analysis

The experimental results were analyzed statistically by carrying analysis of variance (ANOVA) using SPSS 16.0 software (SPSS Inc., USA) to demonstrate the difference between coating treatments, and the calculations were performed at the significance level of α = 0.05. The multiple comparisons between treatments were carried out using the least significant difference (LSD) method.

Table 1. Scoring system for sensory assessment of odor and flavor of fish fillets.

Limit of acceptability	Odor/ flavor	Grade	characteristics	Score
Acceptable	No/off flavor	I	Odor/flavor characteristics of species, very fresh, seaweedy. Loss of odor/flavor. Neutral.	10 9 8 7 6
	Slight off-odors/flavor	II	Slight off-odors/ flavor such as garlic, bready, sour, fruity or rancid.	5 4
Rejected	Sever off odors/flavor	III	Strong off-odors/flavor such as stale cabbage, NH_3, H_2S or sulfides.	3 2 1

RESULTS AND DISCUSSION

1. Chemical assessment

1.1. Change of pH and titratable acidity during storage

Variations in pH and titratable acidity of coated and uncoated loat fish fillets during storage are shown in Fig. (1a) and Fig. (1b) respectively. During 28 days of storage we can notice that the pH values of all treated samples were lower than the pH of control samples because using acids as coatings caused a dramatic drop in the pH starting from the first day for all dipping solutions. The pH value of uncoated control fillets and coated samples was significantly different as confirmed statistically in the multiple comparisons presented in Table 2.

However, after 4 days of storage, the pH of all treated samples increased during this period and this increase in pH corresponds to a decrease in titratable acidity values at 4 days as shown in Fig. (1a) and Fig (1b) respectively. Thereafter, the pH values of all samples decreased again due to denaturation of myofibril protein as a result of conversion of muscle glycogen to lactic acid as reported by Hashimoto and Arai (1985), or due to dissolution of CO_2 in the fish muscle (Meekin, *et al.,* 1982). Several authors have reported a decrease in pH with the increase in the concentration of CO_2 in the storage atmosphere (Lannelongue *et al* 1982; Tiffney and Mills, 1982). Furthermore, an increase in pH was observed again at the end of the storage particularly in control sample (6.94) which may be elucidated to the production of volatile base compounds by bacterial activity (Cann, *et al* 1983).

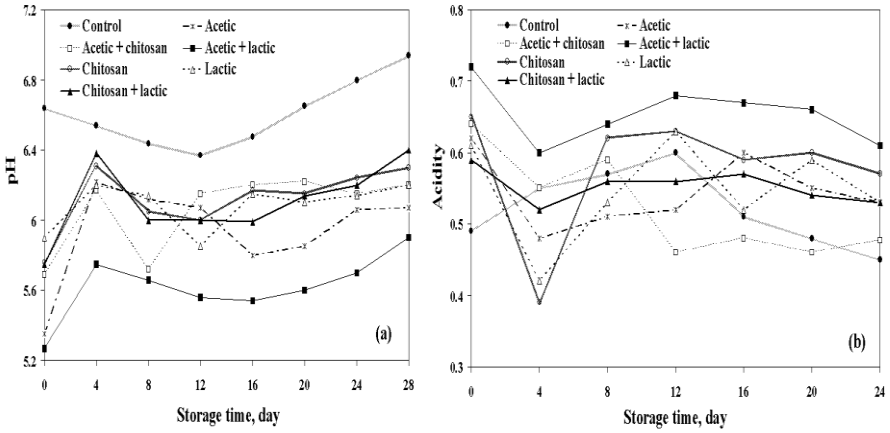

Fig. (1) Effect of different coating solutions on (a) pH and (b) Titratable acidity of vacuum-packed lout fillets during storage at 4°C.

As expected, the titratable acidity of the samples followed a reverse trend with pH as depicted in Fig. (1b). It is evident that samples with higher titratable acidity also had correspondingly lower pH values, confirming that level of pH depended on the amount of acid in the samples; if there is no buffering activity in the packaged samples.

The average chemical properties during the whole storage period (28 days) for uncoated control samples as well as coated fillets with different dipping solutions are tabulated in Table 2. It was evident that the pH of all coated fillets is significantly different ($P < 0.05$) than the pH value of uncoated control samples. Expectedly, fillets coated with acetic and lactic acids together caused a drastic drop in pH which was significantly different ($P < 0.05$) than the other coating solutions. An opposite trend could be easily figured out in case of titratable acidity because their values in case of acetic + lactic treatment were significantly different ($P < 0.05$) from the other treatments.

1.2. Change of TBA during storage

Changes in TBA value as a measure of oxidative rancidity of the lout fish fillets are illustrated in Fig. (2a). The TBA values increased during storage for all fillet samples. Similar observations were observed by several authors (Huang *et al.,* 1994, Josephson *et al.*, 1985; Nolan *et al.,* 1989). In this study, TBA values for coated fillets were below the threshold level of 3 mg of malondialdehyde (MDA)/kg fish muscle (Connell, 1990); meanwhile the TBA values for control fish exceeded this limit at the end of storage. Moreover, the reduction in TBA values of coated fillets compared to control samples during chill storage was remarkable and significantly different ($P < 0.05$) as shown in Table (2). Oxidation of fat is found to increase during cold storage and the rate was reduced by vacuum-packaging and icing (Baldrati *et al.,* 1982).

Table 2. Chemical attributes of lout fish fillets (*Sciaena umbra*) during the whole storage period at 4°C under the effect of different dipping solutions.

Treatments	Chemical attributes (mean±standard deviation)				
	pH	Acidity	TBA	TVB-N	TMA
Control	6.607^{a}±0.191	0.509^{a}±0.061	2.088^{a}±1.173	22.619^{a}±7.969	5.506^{a}±3.509
Acetic	5.942^{b}±0.276	0.541^{a}±0.047	1.486^{bc}±0.849	15.923^{b}±4.190	3.153^{bd}±1.989
Acitic+Chitosan	6.062^{b}±0.222	0.517^{a}±0.068	1.301^{bc}±0.763	14.029^{bc}±4.321	2.912^{cde}±1.952
Acitic+Lactic	5.622^{c}±0.183	0.633^{b}±0.069	1.433^{bc}±0.701	14.410^{bc}±4.105	3.457^{bc}±2.026
Chitosan	6.122^{b}±0.183	0.558^{ab}±0.098	1.263^{bc}±0.732	13.554^{c}±3.302	3.112^{be}±1.911
Lactic	6.085^{b}±0.134	0.531^{a}±0.079	1.165^{c}±0.745	14.015^{bc}±3.068	3.664^{b}±2.126
Chitosan+Lactic	6.107^{b}±0.218	0.540^{a}±0.042	1.531^{b}±0.796	13.028^{c}±3.027	2.987^{be}±1.906

All values are average of eight determinations ± standard deviation. Values within a column with the same letter are not significantly different (p > 0.05).

1.3 Change of TVB-N during storage

TVB-N in fish is mainly composed of ammonia and primary, secondary and tertiary amines. Fish with a level of 35-40 mg TVB-N/100g of fish muscle is usually regarded as spoiled fish (Lakshmanan, 2000). However, a rejection limit of 20 mg per 100 g of fish has been proposed for fatty fish (Sikorski *et al.*, 1990). Values of TVB-N increased in all samples during storage as shown in Fig. (2b). TVB-N contents increased from an initial value of 7.369, 7.369, 6.316, 6.843, 6.316, 7.896 and 7.369 mg TVB-N/100g to 33.98, 20.003, 18.950, 20.003, 16.844, 17.897 and 15.792 mg TVB-N/100g at the end of the storage for control, acetic, acetic +chitosan, acetic +lactic, chitosan, lactic and chitosan +lactic samples. Values of TVB-N in the case of treated fillets samples were found to be significantly ($P < 0.05$) lower compared to control samples as clearly shown in Table 2. Low levels of TVB-N in treated samples were due to either a reduced bacterial population or decreased capacity of bacteria for oxidative de-amination of non-protein nitrogen compounds or both (Banks *et al.,* 1980). In all samples (except control), the values of TVB-N were found to be within the safe limit (<35-40 mg) throughout the storage period.

1.4 Change of TMA-N during storage

TMA-N is also used as an index of quality for deciding the freshness state of fish. The TMA-N contents of fish fillet samples showed an increasing trend with time in case of control (the highest value) as well as in treated fillets as shown in Fig. (2c). The TMA-N contents increased from an initial values of 0.421, 0.526, 0.316, 0.316, 0.632, 0.632, 0.632 mg TMA-N per 100 g muscle to 10.4, 5.754, 5.681, 5.981, 5.381, 6.104 and 5.853 mg TMA-N/100g at the end of the storage for control, acetic, acetic + chitosan, acetic + lactic, chitosan, lactic and chitosan + lactic samples, respectively. Since TMA-N of 10-15 mg/100g fish is considered as the limit of acceptability for human consumption in chilled fish (Connell, 1990; Dalgaard *et al.*, 1993), this

means that all treatments were found to be within the acceptability limit throughout the storage period. However, values of TMA-N showed statistically significant differences between all groups during storage period especially between the coated and uncoated control samples as shown in Table 2.

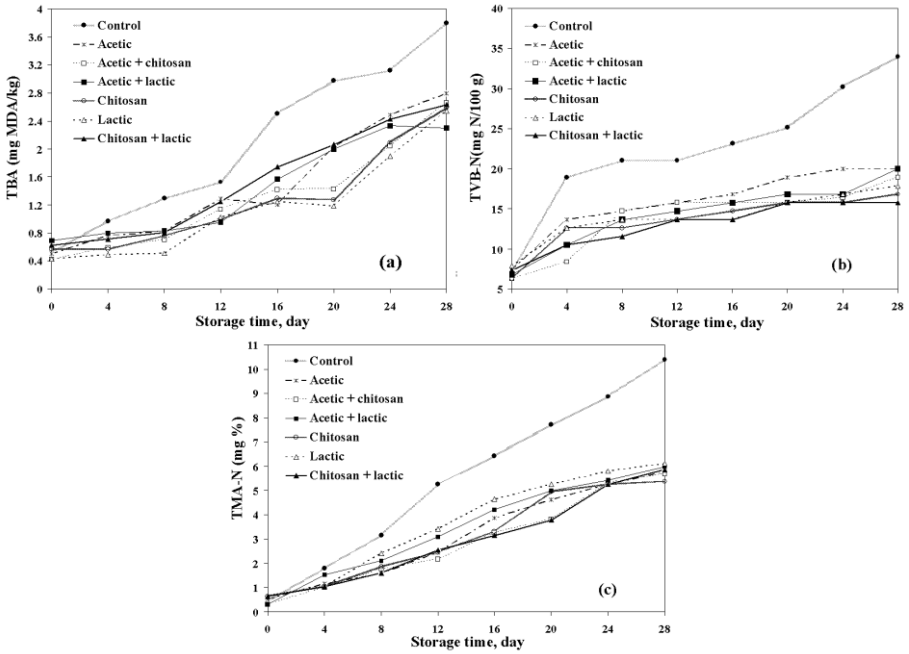

Fig. (2) Effect of different coating solutions on (a) TBA, (b) TVB-N and (c) TMA-N of vacuum-packed fish fillets during storage at 4°C.

2. Microbiological analysis

2.1. Changes in total bacterial count (TBC)

As shown in Fig. (3a), the initial total bacterial counts of all treated samples were less than 4 log CFU g^{-1}, but in case of uncoated control sample it was higher than 4 log CFU g^{-1} which indicates acceptable fish quality, considering the proposed upper limit for aerobic plate count is 5 ×10^5 CFU g^{-1} (5.7 log CFU g^{-1}) for fresh fish (ICMSF, 1986). The number of aerobic bacteria decreased during the first 4 days of storage for all treatments which can be attributed to the inhibitory effect of vacuum packaging and treating solutions at low temperature (4°C). This result is in agreement with Manju *et al.* (2007) who reported that a combination of different factors, namely vacuum-packaging; treatment with preservative (sodium acetate) and storage at refrigerated temperature could be used to prolong the shelf life of pearlspot fish to a great extent. It is emphasized that the success of vacuum-packaging is completely dependent on the initial quality of the fish and on adequate temperature control throughout storage. The concentration of total bacteria at the 8th day of storage increased again but the highest number of TBC was

observed in control at all time of storage. When the aerobic plate count reaches 10^6 CFU per gram or milliliter in a food product, it is assumed to be at, or near, spoilage. (El-Marrakchi *et.al.,* 1990). In this study, only the control samples reached this spoilage limit at day 16; meanwhile all other treated samples were within the acceptable limit throughout the whole storage period. Also, it was obvious to notice that the more effective treatment was chitosan + acetic flowed by chitosan + lactic and then chitosan. This result is due to the fact that gelation by acetic acid results in less susceptibility to microbial spoilage and hence better storage stability in refrigerated storage as reported by Venugopal *et al.* (1994). The amount of absorbed chitosan onto the bacteria cell wall determines the antibacterial activity of chitosan (Loosdrecht *et al.,* 1987; Chen *et al.,* 2002). The more chitosan absorbed would result in greater changes in the structure of the cell wall and in the permeability of the cell membrane of bacteria that results in the death of bacteria.

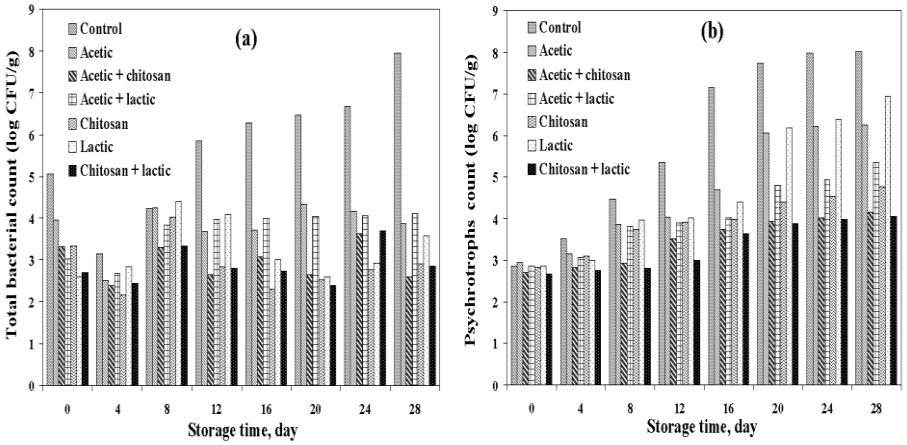

Fig. (3) Changes in (a) total bacterial count and (b) Psychrotrophic bacterial count of vacuum-packed fish fillets under the effect of different coatings during storage at 4°C.

As demonstrated in Fig. (3b), the initial total aerobic psychrotrophs counts were less than 3 log CFU/g for all samples indicating very good fish quality because it is within the acceptable limit (<3 log CFU g^{-1}). Samples of fillets coated with acetic + chitosan, chitosan + lactic and chitosan had psychrotrophic bacterial count of less than 10^5 CFU/g during the entire storage period, whereas uncoated (control), lactic, acetic, and acetic + lactic samples exceeded this level after 12, 20, 20, and 28 days, respectively. Initial bacterial population, gas/fish ratio and packaging materials are important factors affecting shelf life of fish in packages (Pérez -Alonso *et al*., 2004).

2.2. Changes in psychrotrophic and *Pseudomonas* counts

The shelf life of fresh fish is limited by the growth and biochemical activities of gram-negative psychrotrophic strain of *Pseudomonas* with the presence of atmospheric oxygen. These spoilage organisms can be inhibited

by packaging of products in an impermeable film under a CO_2-enriched atmosphere (Huss, 1994). Fig. (4a) shows that low concentration of *Pseudomonas* was observed in case of coated fillets (< 5 log CFU/g) during the entire storage period, whereas uncoated fillets (control) exceeded this limit after only 8 days. That is because vacuum packaging techniques helped in changing the level of oxygen in a food environment which affects the growth of different groups of microorganisms. The removal of O_2 is more important than the inclusion of high CO_2 content in the package, due to oxidation of fat. In general, the efficiency of this effect increased with using the coat.

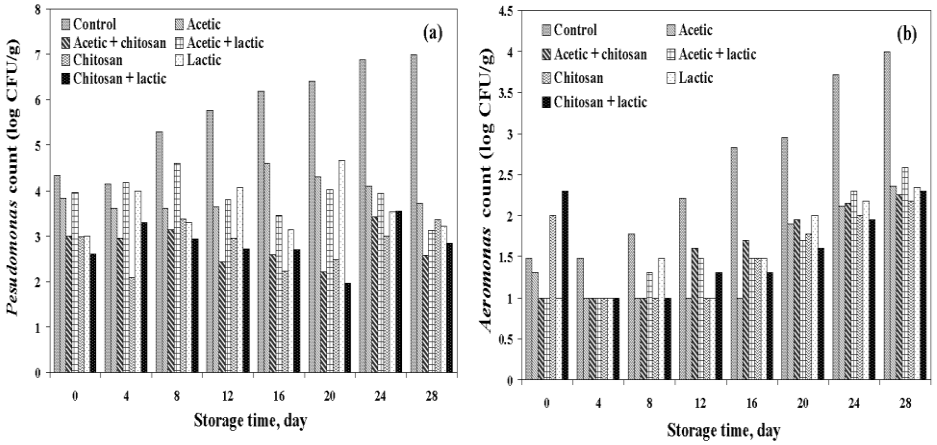

Fig. (4) Changes in (a) *Pseudomonas* count and (b) *Aeromonas* count of vacuum-packed fish fillets under the effect of different coatings during storage at 4°C.

2.3. Changes in *Aeromonas* count

Aeromonas is considered a potential cause of food-borne illnesses. This organism is widely distributed in the environment and is commonly detected in fish. Changes in *Aeromonas* count during storage period are shown in Fig. (4b). The initial total *Aeromonas* counts in all samples were < 3 log CFU/g, and this count continues to be under the acceptable limit for the whole storage period. That because gram-negative bacteria (e.g. *Aeromonas)* are normally more sensitive to lower pH due to using acid solutions in addition to vacuum packaging (Ray and Sandine, 1992). However, growth of *Aeromonas* was detected, but the vacuum packaging conditions inhibited growth compared with air storage (Slade and Davies, 1997).

3. Sensory analysis

Sensory scores of uncoated control fillets and treated fillets with different coating solutions are presented in Fig. (5). Sensory scores showed a significant decline in sensory quality of both control and treated samples with increasing storage period. Fish samples were considered to be acceptable for human consumption until the sensory score reached 4. Beyond this score,

fish spoilage gave rise to the subsequent development of strongly fishy, rancid and putrid odors, and fish was clearly rejected for consumption by the panel. Sensory scores declined from an initial value of 10 to 2, 5, 8, 6, 6, 5 and 6 for control, acetic, acetic +chitosan, acetic +lactic, chitosan, lactic and chitosan +lactic samples, respectively. It was easy to figure out that control samples was rejected after 16 days; meanwhile the other treated samples were acceptable up to 28 days. The statistical analysis shows that all treated samples are significantly different than uncoated control samples in terms of their sensory quality. However, the best treatment in terms of sensory point of view was acetic +chitosan. The use of acetic +chitosan and vacuum-packaging was found to extend the shelf life of fish samples in the present study also.

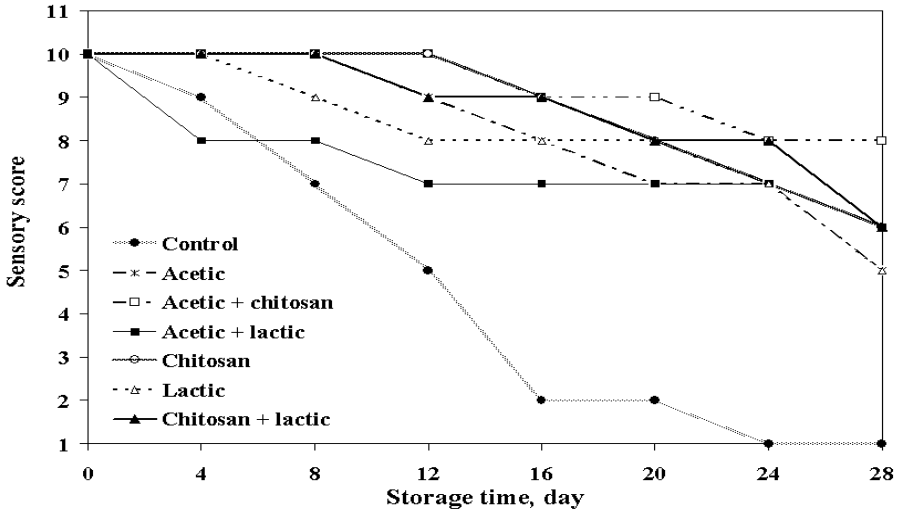

Fig (5) Sensory score of vacuum-packed fish fillets treated with different coatings during storage at 4°C.

CONCLUSIONS

Based on the concept of protection barrier technology, the use of coating may contribute to improve safety in minimally processed fish thereby prolonging its shelf life. Coating may be applied on minimally processed fish, combined with other means, such as quality of raw material, hygienic processing conditions and storage temperatures. The combination of these treatments as barrier offers a greater potential for shelf-life extension of minimally processed fish. The results of the present study revealed that vacuum-packaging alone (control without coating treatment) would not be of much use under the reported experimental conditions. The results from this study clearly suggest that a combination of vacuum packaging, coating with acetic +chitosan and storage at refrigerated temperature (4˚C±1) could be used to prolong the shelf life of Lout fish (*Sciaena umbra*) fillets to a great extent. Also, the results of chemical (pH, TBA, TVB-N), microbiological and

sensory evaluation analyses indicated that acetic +chitosan coating on lout fish can lead to the retention of the good quality characteristics and extension of the shelf life up to 28 days of storage.

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تأثير بعض محاليل الغمر والشيتوزان على جودة شرائح سمك اللوت المعبئة تحت تفريغ

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يهدف هذا البحث الى دراسة تأثير الشيتوزان (١%) كغلاف قابل للأكل وبعض محاليل الغمر مثل (حمض الاسيتك ١% ، حمض اللاكتيك ١%) على جودة شرائح سمك اللوت المعبأة تحت تفريغ خلال الحفظ بالتبريد (4°م ±١). ولهذا الغرض تم مقارنة ٧ معاملات وهى شرائح السمك الخام ، شرائح السمك المغمورة فى ١% الشيتوزان ، حمض أسيتيك ١%، حمض لاكتيك ١%، الشيتوزان١ %+ حمض أسيتك ١% ، الشيتوزان١% + حمض لاكتيك ١% و حمض أسيتك ١% + حمض لاكتيك ١%.

وقد أوضحت الدراسة زيادة العمر التخزينى لشرائح السمك المعبأ تحت تفريغ والمعامل بالشيتوزان والأسيتيك إلى ٢٨ يوم مقارنة بـ ١٢ يوم فقط للكنترول والمعبأ فقط تحت تفريغ دون أى معاملة غمر قبل التعبئة. وكان مخلوط الشيتوزان والأسيتيك هى أفضل المعاملات مقارنة بالشيتوزان بمفرده أو مخلوطاً مع حمض اللاكتيك حيث أعطى أطول فترة حفظ لشرائح السمك (٢٨ يوم) وكانت قيم الثيوباربيوتريك (TBA) لشرائح سمك اللوت المعامل بمخاليط التغطية والتعبئة تحت تفريغ أقل من ٣ مليجرام مالون ألدهيد لكل ١ كيلو جرام سمك (داخل الحدود المسموح بها) بينما تعدت هذه القيمة للكنترول الحد المسموح به ووصلت إلى ٣,٨ مليجرام مالون ألدهيد لكل ١ كيلو جرام سمك . وكانت قيم النيتروجين الكلى المتطاير (TVB-N) لجميع المعاملات داخل الحد المقبول للإستهلاك الآدمى حيث كانت أقل من ٣٥ ـ ٤٠ مليجرام نيتروجين لكل ١٠٠ جرام سمك طوال فترة التخزين فى حين أن الكنترول وصل لحد الفساد خلال ١٦ يوم من التخزين. كما أظهر التحليل الاحصائى وجود فروق معنوية بين شرائح السمك المعاملة والكنترول فى قيم كل من TBA ، TVB-N ، TMA.