Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 - 6131 Vol. 22(2): 41- 49 (2018)
ejabf.journals.ekb.eg

Quality criteria of Mullet fillets (*Mugil cephalus*) storage at 4±1°C under modified atmosphere packaging.

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ARTICLE INFO

Article History:
Received: April 5, 2018
Accepted: May 20, 2018
Available online:June 2018

Keywords:
Mugil cephalus
Mullet fillets
modified atmosphere packaging
Quality criteria

ABSTRACT

This study was conducted to compare the effect of four package systems on quality criteria of mullet's fish fillets stored at 4±1°C for 18 days. Modified atmosphere packaging (MP1, 60% CO_2 \ 35 % N_2 \ 5 % O_2 and MP2, immersing fillets in sodium chlorides solution prior to package with the same condition of MP1) suppressed ($p<0.05$)the growth of total count and psychrophilic bacterial when compared with aerobic (AP) and vacuum package (VP). During storage period aerobic package taken the highest physicochemical, microbial load and the lowest sensory scores. Likewise, MP1 and MP2had lowertotal volatile basic nitrogen, Trimethy lamine nitrogen, Thiobarbituric acid and microbial loadcompared toaerobic package samples ($p<0.05$). However, MP2 showed exudate loss lower than MP1. Thiobarbituric acids of the samples kept VP were lower compared to other condition package in the same time. Overall acceptability of MP1, MP 2 and VPsamples were accepted during storageof fifteen, eighteen and twelve days, respectively. While, aerobic package samples had the acceptability six days only. Therefore, MP2 was a best choice for extending the shelf-life and maintained the quality of mullet's fish fillets.

INTRODUCTION

Fresh fish have become important popular; in the market fresh fish are available. However, fish is perishable food limiting by short shelf-life. Immediately after death, several changes are occuring in fish and fishery products, especially with improper handling. Therefore, new techniques have been applied to maintain the quality criteria of fish and fishery productsDuan *et al*., (2011). Sivertsvik *et al.* (1999) studied the quality of refrigerated (≤1°C) gutted salmon (*Salmo salary*) stored in plastic bags containing 50 % and 100 % CO_2 and 60% CO_2 / 40 % O_2, in addition toconventional packaging material (polystyrene) during transport. The authors noticed that the conventionally packed salmon showed a high microbial load. After 13 days of storage, Modified atmosphere packaging (MAP) salmon showed better overall acceptability compared to aerobic packed one. Shelf-life of refrigerated fish could be extended by using MAP, specifically elevated CO_2 levels, which has shown to reduce the growth of bacteria (spoilage and pathogenic).

Refrigerated fish, including catfish and sea bass packed with CO_2 had 40-100 % increase in stability, mainly due to an extension in the lag phase and retard logarithmic phase of organisms (Maqsood and Benjakul, 2010; Provincial *et al*., 2010and Masniyom *et al*., 2 011).

The aim of any packaging system for fresh foods is to reduce or retard undesirable changes to the sensory parameter. Consumer rejection of this product when deterioration in the quality criteria occurs, results in economic losses. Therefore, a preservative packaging should ideally inhibit undesirable enzyme and non-enzymatic reactions. (Ščetar*et al*, 2010 and Ulusoy and Özden, 2011). VP and MAP with refrigeration have received increasing attentions method of food preservation (Masniyom, 2011and Noseda *et al*., 2012).

MAP is known as the preservation method by changes the percentage of gases (single or a mixture) around commodities (Arashisar *et al*., 2004 and Del Nobile *et al*., 2009). MAP is used to protect the commodities from microbial growth and retard the physiochemical parameters in addition to maintain the sensory attributes. These values reflect to the judgments of purchasers as fresh commodities are preferred more than those frozen or processed (Goulas and Kontominas, 2007). MAP can maintain andextend the storageperiod of food. There are many factors affected on the microbial growth during storage such as the type of the product, materials of packaging, appropriate and ratio gas composition, storage temperature and hygienic practice during processing and packaging. Fishery products packed with high concentration of carbon dioxide more effective compared to VP in reducing the growth of spoilage and pathogenic bacteria (Velu *et al.,* 2013).

In fishery products to increment the water holding capacity, retard the growth of bacteria and reduces the oxidation of unsaturated fatty acids phosphate compounds have been used (Etemadian *et al*., 2012 and Özpolat and Guran 2015). Tilapia fish pretreatment with 2% solution of sodium tripolyphosphate combined with MAP (90% CO_2+10 % O_2) was more effective in reduction of microbial load and maintain quality of fish (Abouel-Yazeed, 2013). Precooked white shrimp coated withessential oil prior to packing in MAP (60% CO_2: 40 % N_2) during cooling storage causes extension the storage period to 28 daysbased on odor test (Teerawut *et al.,* 2016). Dipping fish in sodium chloride solutions preserves the texture and color when combined with MAP storage (Mitsuda *et al.,* 1980). Dipping in NaCl plus MAP markedly reduced exudation and increment water holding capacity in comparison with MAP. Sodium chloride dipping also significantly increased the shelf-life plus two days compared to MAP-stored samples were refused after14 days of storage. MAP-stored samples were refused after one week of control samples (Pastoriza*et al.,* 1998).

The objective of this study was to investigate four different package systems (modified atmosphere packaging or MAP (MP1 and MP2), vacuum packaging or VP, and aerobic packaging or AP on the quality criteria of mullet fish fillets stored at 4±1°C for 18 days.

MATERIALS AND METHODS

Materials

About 24 kilograms fresh mullet fish (*Mugil cephalus*) average 340±30g used as research material were purchased in the morning from the wholesale fish market in Giza, Egypt during January 2018. Fish were transferred to the laboratory in an ice boxcontaining flaked ice (ice/fillets weight ratio 2:1) within 30 min.The fish samples

were beheaded, gutted and washed gently with tap water, then filleted and skinnedmanuallyusing a sterile scalpel and forceps.The fillets were divided into four lots (36 fillets in each)and packaged as follows: aerobic packaged, vacuum packagedand MAP include: (MP1: 60% CO_2, 35% N2, 5% O_2 and MP2: fillets immersing in 2% sodium chlorides solution for 5 min prior to packed under the same condition in MP1). All samples were packaged in the foam tray capacity of each 2 fillets, then packaged with polyamide / polyethylene bags and kept after them in therefrigerator (4±1^{O} C). Polyamide / polyethylene (PA/PE) packagingrolls were obtained fromTecno-plast Company, Bourg El-Arab, Cairo.The above packaging materials were used for preparation of packages of 30×20 cm.Modified atmosphere packaged mullet fish fillets were sealed by a Model Witt Oxybaby headspace Gas analyzer (O_2, CO_2, and N_2) company Saguenay group wittgas. Thegas ratio was 60% N_2; 35% CO_2 and 5% O_2, typical for packing fatty fish in MAP (Ibrahim *et al*., 2008).The ratio (gas/sample) in all pouches was 2:1 (v/w) for MAP conditions. Three pouches were taken for each analysis from each batch. Sodium chloride: Salt fine refined (El-Nasr Co.) was purchased from local markets, Nasr City, Cairo, Egypt.

Physicochemical analysis:

Exudates loss was measured as the percentage loss of weight mullet fish fillet compared with the initial weight (Pastoriza *et al.,* 1996). Total volatile basic nitrogen (TVB-N) was determined by the Macro distillation method proposed by Pearson (1991). Trimethylamine nitrogen (TMA-N) was determined as described by the A.O.A.C (2002). Thiobarbituric acid values (TBA) were determined spectroph-otometrically according toPearson's description (1991).

Microbiological analysis

10 g of fish muscles was suspended in 90 ml sterile saline (0.85% NaCl). Serial decimal dilutions were prepared and 1ml samples of appropriate dilutions were poured on selectivemedium for analyzing the microbial profile using standard procedures (APHA, 1992) for total bacterial count (TBC) (30°C, 3 days) and Psychrophilic bacteria (PCB) (7°C, 10 days) on plate count agar. The results were expressed as log10cfu/g of sample.

Sensory Evaluation

The organoleptic quality attributes (appearance, flavor, texture and overall acceptability) of untreated and treated mullet fillets by cooked samples (the samples were wrapped with aluminum foil, cooked in steaming pot until the core temperature of each sample reached 70^{o}C. Cooked samples were left to drain and allowed to cool at room temperature). The panel consisted of six members were evaluated at zero time and periodically every three days, according to the procedure of (Fey and Regenstein, 1982) using the following numerical system: excellent 8.5< 10, very good 7.5< 8.5, good 6.5< 7.5, accepted 5.0<6.5, poor4.5<5.0 and very poor 0<4.0.The evaluation of odour was carried out at the moment of opening the pack.

Statistical Analysis:

All data (n = 3) were subjected to Duncan's test (P<0.05) to evaluate the effect of MAP and storage condition in this study on physicochemical, microbial and sensory of mullet fillets stored at 4±1 °C periodically at 3 days interval for 18 days.. SPSS version 20.0 was used for statistical analysis.

RESULTS AND DISCUSSION

Physicochemical Quality Parameters

Exudates loss was found to be zero for aerobic-packaged mullet fish fillets onthe 3th day, and significantlyincreased (P < 0.05) to 0.03 on the 6th day when rejected samples occurred (Table 1). The VP samples had no exudates on the 6th day and significantly increased and reached the value of 0.4% on the 12th day of storage.

An increase in exudates loss was noticed in all samples with increasing storage time ($p < 0.05$).MP1 showed that exudate values were significantly ($p<0.05$) higher than MP2, may be du to the result of immersing the fillets in NaCl before MAP storage which reduced exudate loss significantly ($p<0.05$).Carbon dioxide causes acidity in fish muscle, results reduce the capacity of fish proteins to hold water (Cheftel and Cheftel, 1976).Immersing fish in NaCl solutions before MAP reduced the exudate loss (Pastoriza *et al*., 1998).The negative effect of exudate loss could be overcome by the use an adsorbent pad on the package (Wang *et al.* 2008)and /or previously immersing fish in NaCl solutions before MAP.

Table 1: Physicochemical parameters of mullet fillets stored at 4±1°C under different packaging conditions.

Storage Period (days) / Packed	Storage Period (days)						
	0	**3**	**6**	**9**	**12**	**15**	**18**
Exudate loss							
AP	0.0±0.0^{a}	0.00±0.0^{a}	0.03±0.01^{a}	R	R	R	R
VP	0.0±0.0^{a}	0.00±0.0^{a}	0.00±0.00^{a}	0.1±0.02^{a}	0.40±0.03^{b}	R	R
MP1	0.0±0.0^{a}	0.01±0.001^{a}	0.68±0.18^{b}	0.74±0.1^{b}	0.95±0.15^{c}	1.62±0.17^{d}	R
MP2	0.0±0.0^{a}	0.00±0.00^{a}	0.45±0.15^{b}	0.63±0.08^{b}	0.73±0.12^{b}	1.21±0.05^{c}	1.74±0.40^{d}
TVB - N							
AP	10.1±0.3^{a}	18.7±0.2^{b}	29.4±0.3^{c}	R	R	R	R
VP	10.1±0.3^{a}	13.9±0.6^{a}	20.7±0.2^{b}	24.3±0.7^{b}	28.8±0.5^{c}	R	R
MP1	10.1±0.3^{a}	13.6±0.5^{a}	17.2±0.2^{a}	23.1±0.6^{b}	26.7±0.3^{c}	29.3±0.7^{c}	R
MP2	10.1±0.3^{a}	12.8±0.6^{a}	15.6±0.5^{a}	20.4±0.8^{b}	24.5±0.4^{b}	27.9±0.3^{c}	29.5±0.5^{c}
TMA- N							
AP	4.9±0.12^{a}	7.4±0.8^{b}	9.4±0.14^{c}	R	R	R	R
VP	4.9±0.12^{a}	6.2±0.8^{b}	7.3±0.15^{b}	8.1±0.07^{c}	9.3±0.22^{c}	R	R
MP1	4.9±0.12^{a}	5.8±0.19^{a}	7.1±0.26^{b}	8.2±0.8^{c}	8.7±0.20^{c}	9.5±0.17^{d}	R
MP2	4.9±0.12^{a}	5.4±0.25^{a}	6.7±0.16^{b}	7.2±0.33^{b}	7.8±0.6^{c}	8.5±0.11^{c}	9.6±0.24^{d}
TBA							
AP	0.76±0.23^{a}	1.22±0.11^{b}	1.84±0.34^{c}	R	R	R	R
VP	0.76±0.23^{a}	0.94±0.34^{a}	1.12±0.12^{a}	1.42±0.46^{b}	1.63±0.56^{c}	R	R
MP1	0.76±0.23^{a}	1.24±0.32^{b}	1.52±0.61^{b}	1.84±0.19^{c}	1.97±0.51^{d}	2.14±0.66^{d}	R
MP2	0.76±0.23^{a}	1.15±0.41^{a}	1.36±0.33^{b}	1.75±0.29^{c}	1.82±0.31^{c}	1.96±0.27^{d}	2.09±0.63^{d}

Data are presented as means ± standard deviation (SD). Means followed by different letters in each column are significantly (P<0.05) different. R: Rejected. AP: aerobic packaged, VP: vacuum packaged. Modifiedatmosphere packaged (MP1, MP 2).

TVB-N of aerobic condition increased significantly ($p<0.05$) throughout all storage periods. Both MAP stored fish fillets showed TVB-N values significantly lower ($p<0.05$) than aerobic conditionand vacumpackage on the 6th day cold storage. TVB values of AP were two-fold higher than MP2 on the 6th day cold storage. Consequently, NaCl also reduced TVB values significantly ($p<0.05$) during all storage periods.

Concerning 30mg/100gTVB-N the maximum limit of acceptability for consumption of fish, mullet fish fillets would have a shelf-life of 6 days, which would be extended to 12 days when stored under vacum, to 15 days under MAP only and 18 days when immersing in Na cl before MAP storage at 4±1°C. Similar situation occurred in TVB-N levels reported by Rodríguez *et al.* (2006) and Santos *et al*. (2013) in turbot stored in refrigerated condition and Teerawut *et al.* (2016) in white shrimp kept in cold storage.

TMA-N is related with the off-odor of the spoiled fish and used as indicator of deterioration fish by bacterial activity (Huss, 1995). TMA-N content in samples kept in APincreased significantly ($p<0.05$) throughout all storage periods, while it slightly increased in MAP(MP1 and MP2) and VP (table1).In general, MAP causes higher

retard in TMA-N formation.The slow rate of TMA-N production in MAP stored samples attributed to an inhibition of microorganisms, by CO_2-enriched atmosphere (Ruiz-Capillas and Moral, 2001). TMA-N content of mullet fillets kept aerobic packaging reached 9.4mg/100 g after six days, while, vacuum packaging samples and MAP,(MP1and MP2) reached 9.3 mg/100 g after twelve,9.5 mg/100 g after fifteen and 9.6mg/100 g after eighteen days of storage, respectively. Our result was parallel withRavi-Sankar *et al.* (2008) and Masniyom *et al*. (2013).

TBA values in mullet fillets packed with different conditions are presented in (table1). TBA value was increased significantly ($p<0.05$) throughout all storage periods, indicating that lipid oxidation occurs during storage. However, VPsamplesshowed lower TBA, compared with MAP (MP1 andMP2) during storage. This indicated that VP improves the sensory properties in fish by preventing the oxidative rancidity. The result was parallel with Masniyom *et al*., (2013).

Microbiological analysis

Table (2) shows the changes in total plate counts (TPC) and psychrotrophic bacteria counts during storage of mullet fillets. Both TPC and psychrotrophic bacteria counts increased significantly ($p <0.05$) with time of storage increase. The initial TPC and psychrotrophic counts were 3.54 and 2.35 log cfu/g, respectively. The initial TPC was around 4.0 log cfu/g, higher than the psychrotrophic by about 1 log number in all samples. Therefore, TPC and psychrotrophic can be considered as indicative of hygiene conditions.The initial TPC and psychrotrophic found in this study was in agreement with (Che Rohani*et al*., 2008). TPC and psychrotrophic counts of mullet fillets in aerobic packed rapidly increased to 7 Log CFU/g after six days and were generally higher than that of VP samples and MAP (MP1 and MP2) ($P < 0.05$).In six days of storage, TPC in mullet fillets packed in aerobic condition, VP and MAP (MP1and MP2) were5.89, 3.26, 2.74 and 2.61, respectively.

Table 2: Microbial load of mullet fillets stored at 4±1°C under different packagingconditions.

Storage Period (days) / Packed	Storage Period (days)						
	0	**3**	**6**	**9**	**12**	**15**	**18**
Total plate count (log10 CFU/g)							
AP	3.54±0.23^{a}	4.20±0.19^{b}	5.89±0.32^{c}	R	R	R	R
VP	3.54±0.23^{a}	2.25±0.08a	3.26±0.45a	4.52±0.14b	5.93±1.85c	R	R
MP1	3.54±0.23^{a}	2.09±0.52a	2.74±0.15a	3.63±0.27a	5.34±0.65b	5.90±0.28c	R
MP2	3.54±0.23^{a}	1.96±0.11a	2.61±0.26a	3.35±0.79a	4.83±0.21b	5.26±0.43b	5.88±0.27c
psychrophilic count (log10 CFU/g)							
AP	2.35±0.22^{a}	2.64±0.27^{a}	5.87±0.29^{b}	R	R	R	R
VP	2.35±0.22^{a}	2.68±0.12^{a}	3.50±0.23^{a}	4.36±0.16^{b}	5.79±0.53^{c}	R	R
MP1	2.35±0.22^{a}	2.51±0.37^{a}	3.34±0.12^{a}	3.82±0.37^{a}	4.25±0.14^{b}	5.94±0.34^{c}	R
MP2	2.35±0.22^{a}	2.47±0.41^{a}	3.12±0.36a	3.20±0.54^{a}	3.93±0.42^{b}	4.02±0.18^{b}	5.90±0.23^{c}

Data are presented as means ± standard deviation (SD). Means followed by different letters in each column are significantly ($P<0.05$) different. R: Rejected. AP: aerobic packaged, VP: vacuum packaged. Modified atmosphere packaged (MP1, MP2).

While psychrotrophic counts in the same packed and time were5.87, 3.50, 3.34 and 3.12, respectively. VP, MP1 and MP2 (immersing fillets in NaCl prior to MAP) was typically below 7 Log CFU/g and remained at this level during storage of twelve, fifteen and eighteen day, respectively. ICMSF (1986) suggested that TVC of fresh water and marine species exceeded the value of 7 Log CFU/g, which is recommended as the upper acceptability limit.MP1 showed TBC values significantly higher ($p<0.05$) than MP2 (immersing fillets in NaCl prior to MAP). Consequently, NaCl and CO_2-enriched atmospherefurther inhibited bacterial during storage period.

This was probably because CO_2 entered into mass action equilibrium for enzymatic decarboxylation, leading to inhibition of the metabolic activity of microbial flora as result of an extension in lag phase and a reduction in logarithmic phase of spoilage bacteria (Ozogul *et al.*, 2004; Masniyom *et al*., 2011and 2013). The results indicated that immersing mullet fillets in Nacl prior to MAP was more effective in reducing the microbial load on the fish fillets.

Sensory evaluation

Changes in sensory parameters for mullet fillets packaged in aerobic condition, vacuum package and MAP (MP1 and MP2) stored at 4±1°C are shown in (Table 3). Aerobic packaged -stored mullet fillets were sensorial rejected after 6 days storage as evidenced by strong fishy and putrid odor. However, vacuum package accepted for 12 days. While, MAP (MP1and MP2) accepted for a longer period, extending up to 15 and 18 days of storage, respectively. There was a significant reduction ($P < 0.05$) in sensory parameters of all samples with the storage period increased. Our results indicated that keeping the mullet fillets under vacuum and MAP (MP1and MP2) effectively extended the storage period of mullet fillets with high acceptability, especially, in MP2 (immersing the fillets in NaCl prior to MAP). The result was parallel with Masniyom, *et al.* (2005 and 2013) and Abouel-Yazeed, (2013).

Table 3: Sensory evaluation of mullet fillets stored at 4±1°C under different packaging conditions.

Organoleptic Quality Criteria	**packed**	**Storage Period (days)**						
		0	**3**	**6**	**9**	**12**	**15**	**18**
Appearance	AP	9.6	7.5	5.6	R	R	R	R
	VP	9.6	8.6	7.7	6.8	5.5	R	R
	MP1	9.6	8.7	7.9	7.2	5.9	5.1	R
	MP2	9.6	8.9	8.1	7.4	6.8	5.7	5.2
Flavor	AP	9.5	7.3	5.4	R	R	R	R
	UV	9.5	8.5	7.6	6.6	5.4	R	R
	MP1	9.5	8.6	8.0	7.4	6.2	5.3	R
	MP2	9.5	8.9	8.2	7.5	6.9	5.8	5.3
Texture	AP	9.4	7.4	5.5	R	R	R	R
	VP	9.4	8.7	7.8	6.7	5.6	R	R
	MP1	9.4	8.6	7.8	7.4	6.2	5.3	R
	MP2	9.4	8.8	8.2	7.5	6.7	5.9	5.4
Overall Acceptability	AP	**9.5 ± 0.14^{a}**	**7.4 ± 0.12^{b}**	**5.5 ± 0.32^{c}**	**R**	**R**	**R**	**R**
	VP	**9.5 ± 0.14^{a}**	**8.5 ± 0.33^{a}**	**7.7 ± 0.19^{b}**	**6.7 ± 0.25^{c}**	**5.4 ± 0.49^{d}**	**R**	**R**
	MP1	**9.5 ± 0.14^{a}**	**8.6 ± 0.37^{a}**	**7.9 ± 0.39^{b}**	**7.3 ± 0.45^{c}**	**6.1 ± 0.25^{c}**	**5.2 ± 0.16^{d}**	**R**
	MP2	**9.5 ± 0.14^{a}**	**8.9 ± 0.41^{a}**	**8.2 ± 0.26^{b}**	**7.5 ± 0.58^{b}**	**6.8 ± 0.17^{c}**	**5.8 ± 0.24^{c}**	**5.3 ± 0.40^{d}**

Data are presented as means ± standard deviation (SD). Means followed by different letters in each column are significantly (P<0.05) different. R: Rejected. AP: aerobic packaged, VP: vacuum packaged. Modified atmosphere packaged (MP1, MP2).

CONCLUSION

Shelf-life of mullet fillets can be extended by using vacum package and MAP (MP1 andMP2).While, MP2 (immersing fillets in NaCl prior to keeping in MAP) can maintain the quality criteria and extend theshelf-life by three days compared to MP1. On the other hand, VP-stored samples were refused after twelve days and aerobic packaged samples were refused after six days of storage.

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ARABIC SUMMARY

دلائل الجودة لشرائح سمك البورى المعبأ فى جو معدل والمخزن على درجة حرارة التبريد (٤±١درجه مئوية**)**

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اجريت هذه الدراسه لمقارنة تأثير أربعة معاملات تعبئة على جودة شرائح سمك البورى المخزن على درجة حرارة تبريد (٤±١ درجه مئوية) خلال فترة التخزين لمدة ١٨ يوم .

أثبتت الدراسة أن المعامله الأولى (التعبئة فى جو معدل يحتوى على ٦٠% ثانى اكسيد الكربون، ٣٥% نيتروجين، ٥% أوكسيجين) و المعاملة الثانية (وهى غمر شرائح سمك البورى فى محلول من كلوريد الصوديوم ٢% لمدة ٥ دقائق ثم التعبئة فى جو معدل يحتوى على نفس النسب كما تم فى المعاملة الاولى) تثبط نمو الأعداد الكلية للبكتريا وكذلك البكتريا المحبة للبروده وذلك عند مقارنتها بالتعبئة تحت تفريغ وكذلك التعبئة فى الظروف العادية .

أثناء فترة التخزين: العينه الكنترول ارتفعت بها معايير الجوده الفيزوكيمائية وكذلك الميكروبية بينما إنخفضت بها الخواص الحسية، فى حين المعاملة الأولى والثانية إنخفضت بها القواعد النيتروجنية الكلية المتطايرة ومركب الثلاثى ميثيل أمين ورقم حمض الثيوباربتيوريك وكذلك الحمل الميكروبى مقارنة بالمعامله الكنترول .الفقد المائى إنخفض فى المعاملة الثانية مقارنة بالمعاملة الأولى ورقم حمض الثيوباربتيوريك يكون منخفض فى معاملة التعبئة تحت تفريغ مقارنه بطرق التعبئة الأخرى .

درجة القبول العام لشرائح سمك البورى كانت مقبوله عند ١٢ ,١٥ , ١٨ يوم فى معاملة التعبئه تحت تفريغ و التعبئة فى جو معدل وعند غمر الشرائح فى محلول ملحى قبل التعبئة فى جو معدل على التوالى . فى حين أن العينه الكنترول ظلت صالحة لمدة ٦ ايام . ومن هنا تجدر الاشارة إلى أن غمر الشرائح فى محلول ملحى قبل التعبئة فى جو معدل هى أفضل اختيار للمحافظة على دلائل الجودة وإطالة فترة الصلاحية لشرائح سمك البورى .