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Gamma irradiation prolongs the sea bass (*Dicentrarchus labrax* L.) storage and delays the lipids membrane degradation

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Abstract: Mediterranean sea bass (*Dicentrarchus labrax* L.) fish were gamma irradiated at doses 0, 0.5, 1, 2 or 3 kGy and stored at 1°C for 21 days in the dark to assess whether the delay of fish alteration by irradiation involved the protection of membrane lipids. Total basic volatile nitrogen (TVB-N) and the trimethylamine (TMA) contents, the pH, and the lipid composition of membrane were determined in the muscle tissue during storage. The TVB-N and TMA contents and the pH of irradiated and non-irradiated samples increased during storage whereas the external quality decreased in correlation with a reduction in the amount of phospholipids (PL). The degree of unsaturation of PL and of free fatty acids (FFA) fractions decreased, whereas, the ratio of sterol to PL increased. The catabolism of PL was delayed by irradiation during storage as compared to the untreated sample and it was always positively correlated with the total viable counts (TVC) of fish muscle which was also reduced by the treatments. We conclude that the delay of alteration of sea bass tissue during storage by irradiation involved probably a protection of membrane lipids from degradation which seemed to be partially reliable to the micro-organisms load of muscle tissue.

Keywords: Gamma Irradiation, Phospholipids, Sea Bass, Storage

1. Introduction

Because of their nature, fishes become after captures the sit of degradation process particularly rapid due to the fact of the action of endogenous enzymes and bacteriums, and processing in post-crop in these senses was always necessary to insure a best preservation of the product [1-3]. Food irradiation is a food processing technology that exposes certain types of food to a source of ionising energy. Irradiation could have substitute efficiently traditional processes used to preserve the sea products such that chemical treatments basis on glucose oxidase and sorbate of potassium that have an action more selective and limited on the microbial flora, and that can farther damages to products [4, 5]. Gamma irradiation at low doses (0.5-3 kGy) is a process which has potential for shelf-life extension and hygienization of fishery product without too allocating their general aspect [2-6]. Indeed, it was used efficiently in the control of the proliferation of microorganisms, and the alteration of shrimp, fine eagle, cod and carp [6-10].

Although beneficial effects of irradiation on the conservation of several species of sea products were well demonstrated, few works were concerned the sea bass despite its interesting nutritional qualities. This product is one of the more appreciated marines species farmed in many Mediterranean countries to meet the increasing demands on the part of consumers for fresh fish [11]. Ôzden *et al.* [11], based on sensory, chemical and microbiological evaluation, have reported that the quality and shelf-life of aqua-cultured sea bass irradiated at 2.5-5 kGy and stored in ice storage were prolonged compared to untreated samples. Despite theses obviousness, the effects of irradiation on membrane lipids alteration of sea products during storage were not demonstrated although the degradation of phospholipids in post-mortem has been shown [12-16]. This alteration can be considered as a succession of biochemical events and/or of microbiological effects. These changes in the structure of the membrane phospholipids lead to the loss of membrane polarity and therefore membrane fluidity and integrity. The loss of membrane integrity leads to induce the leakage process and consequently faster the fish quality deterioration [15, 17].

The aim of this study was to evaluate the effects of several doses of irradiation on the preservation of the membrane lipids from degradation during storage of sea bass at 1°C and consequently on its quality.

2. Materials and Methods

2.1. Samples Preparation, Irradiation and Storage

Mediterranean sea bass (*Dicentrarchus labrax* L.) fish, captured in less than 12 hours have an average weight of 120 g was provided by a producing place located in the Bizerte region, in north Tunisia. They were rapidly iced and arranged in polyethylene isolated boxes (25-kg capacity) to slow their alteration and to preserve the composition of their microbial flora. Fish selected for integrity, size and freshness were placed by 3 in containers of 250 mL and wrapped with Cellophane paper under aerobic condition.

Irradiation was done the same day of capture following the packaging under crushed ice using a carrier-type irradiator that contains cobalt 60 at the National Center of Nuclear Sciences and Technologies, Tunis, Tunisia, at room temperature, at doses of 0, 0.5, 1, 2 or 3 kGy. The levels of doses were chosen after the preliminary tests showed a delay of external aspect degradation below 3 kGy. Irradiation dose was measured using PMMA dosimeters (PMMA Instruments, Harwell, UK). Following irradiation, fishes were stored during 21 days in the dark at 1 ± 1 °C.

2.2. Criteria of Deterioration

The deterioration of fish was evaluated through the determination of the total basic volatile nitrogen (TVB-N) and the trimethylamine (TMA) contents, the pH and by the evaluation of external fish aspect.

The method used for the dosage of TVB-N is that described by Civera *et al.* [1]. It implies a distillation of a homogenate of 30 g of muscles in 90 mL of perchloric acid (0.6 N) after filtration on Whatman paper rendered basic by addition of 10 mL of magnesium oxide (1/5, p/v), fixed in a boric acid solution (4%) then titrated with H_2SO_4, 0.1N.

The dosage of TMA was made according to the method described by Watabe *et al.* [18]. An extract from trichloroacetic acid (20%) was added to a homogenate prepared with 20 g of muscles and was reacted in Conway cell with boric acid. The formaldehyde (0.1 mL) was added to reduce the parallel reactions. Incubation was made during two hours at 37°C after added 1 mL of K_2CO_3 (112%). The titration was made with the HCl, 0.1N.

The pH of samples was determined according to the method of Benjakul *et al.* [19]. Sample (5g) was mixed with 50 ml of deionised water (w/v) and the mixture was homogenised at 11.000 rpm for 1min using a homogenizer. The pH of homogenate was measured using a pH-meter (model 15, Fisher Scientific, Arvada, Colo., USA) that had previously standardized to pH 4 and 7.

The sensory assessment of fish was evaluated visually on 3 fishes of each cluster at days 0, 7 and 21 by at least five of six of regular panellists, each of whom was trained in fish quality assessment. The scoring was made according the scale 0 to 9, where 0 represented best quality and 9 indicated poorer quality.

2.3. Extraction and Analysis of Lipids

To verify the effect of irradiation on the cellular membrane stabilisation, we have evaluated the phospholipids (PL), free sterol (FS) and free fatty acids (FFA) during fish's storage.

At the end of storage, fishes were fixed in boiling water for 3 min to inactivate endogenous phospholipases. Total lipids were extracted from the tissue using the procedure of Blight and Dyer [20].The lipids in the chloroform phase were separated by TLC on 250 μm silica gel G plates (Fisher Scientific Co., Ottawa, ON). Hexane: diethyl ether: acetic acid (80:20:1; v/v) was used to separate the neutral lipids and chloroform:methanol:acetic acid:water (80:15:15:3,5, v/v) was used to separate the PL. The lipids were visualised briefly in iodine vapours and identified using authentic standards (Sigma). The area corresponding to each class on the TLC plate was scraped into a test tube and transmethylated directly onto the silica gel with 14% (w/v) BF_3 in methanol [21]. For quantitative determination of FA, a known amount of heptadecanoate (C17:0) was added as an internal standard. Methyl esters of FA were analysed by GLC on 30-m capillary DB 225 column (J & W Scientific, Rancho Cordova, CA) as described by Makhlouf *et al.* [22]. FS were silylated directly on the silica gel [23] and assayed by GLC (Hewlett-Packard model 5890A, Mississauga, Canada) using cholestane as a standard. Sterol trisylil derivatives were separated by GLC on a 25-m ULTRA 1 capillary column (Hewlett-Packard).

2.4. Microbiological Analysis

Microbiological analyses were performed on the muscle tissue as described by Özogul *et al.* [24]. Fish sample (10g) was aseptically withdrawn and suspended in 90 ml of sterile 0.1% peptone water and homogenized for 60 s using a laboratory blender stomacher. Further decimal dilutions were made, and then 0.1 ml of each dilution was piped onto the surface of plate count agar (PCA, Difco) plates in triplicate for estimate the total viable counts (TVC). They were then incubated for 2 days at 30°C.

2.5. Statistical Analysis

Analysis of variance of results was made following a factorial randomly complete block design [25] by the GLM procedure of the SAS statistical package [26]. Homogeneity of variance was verified by the standard Bartlett test [27]. Statistical comparison was based on three samples for each treatment. The experiment was repeated twice and only results of seconds are presented.

3. Results

3.1. Effect of Irradiation on the Fish Alteration

The initial TVB-N average values in whole fish were below 2.6 mg per 100 g of fresh weight indicating the freshness of the initial product. TVB-N level increased significantly during storage at 1°C for all samples ($P < 0.001$; Fig. 1). However, this increase varied according to the dose of irradiation. Indeed, it was rapid for control, middle for 0.5 kGy, and lowest for 1, 2 and 3 kGy. Irradiation effect on this criterion was observed immediately after the application.

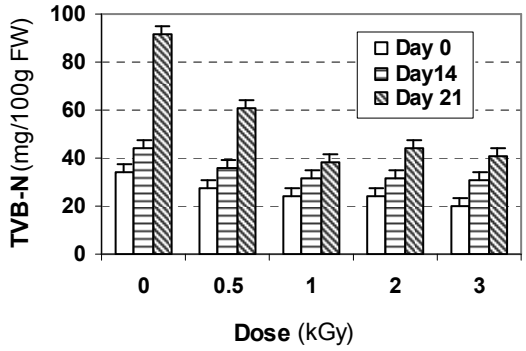

Figure 1. *Change with time of total basic volatile nitrogen (TVB-N) of sea bass (Dicentrarchus labrax* L.*) muscles, irradiated by gamma rays at doses 0, 0.5, 1, 2 or 3 kGy and stored during 21 days at 1°C. Values are expressed as means ± SD for n= 3.*

The initial TMA content in whole fish was as low as 1 mg per 100 g of fresh weight indicating also the freshness of the initial product. TMA level increased significantly for all treatments during storage of sea bass ($P < 0.001$; Fig. 2). This increase was low for irradiated fish and high for control. Irradiation effect on this parameter was not observed immediately after the application.

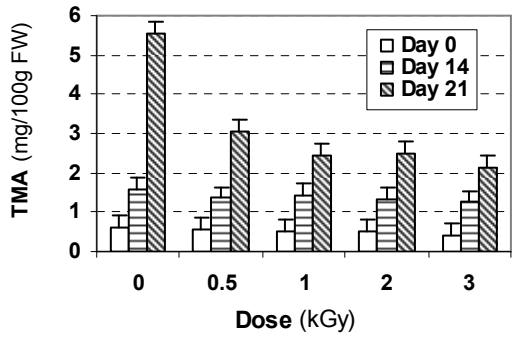

Figure 2. *Change with time of trimethylamine (TMA) of sea bass (Dicentrarchus labrax* L.*) muscles, irradiated by gamma rays at doses 0, 0.5, 1, 2 or 3 kGy and stored during 21 days at 1°C. Values are expressed as means ± SD for n= 3.*

The pH of fish increased significantly during storage of sea bass for all treatments ($P < 0.001$; Fig. 3). Nevertheless, the irradiation lowers this increase. No effect of irradiation on this criterion was observed just after the application.

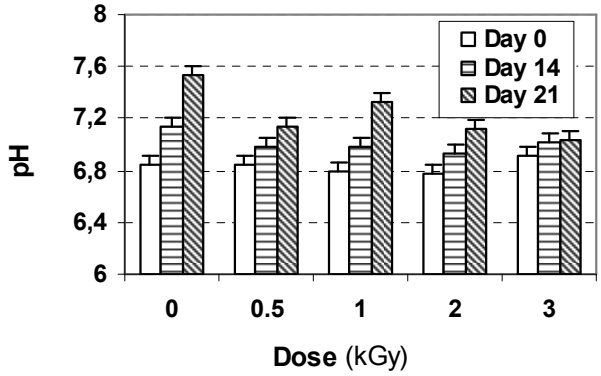

Figure 3. *Change with time of pH of sea bass (Dicentrarchus labrax* L.*) muscles, irradiated by gamma rays at doses 0, 0.5, 1, 2 or 3 kGy and stored during 21 days at 1°C. Values are expressed as means ± SD for n= 3.*

The external fish quality decreased significantly during storage of sea bass for all treatments ($P < 0.001$; Fig. 4). Fish irradiated at 2 and 3 kGy seemed to keep an acceptable appearance until the end of the experiment. The irradiation effect on the external fish quality was not observed at day 0.

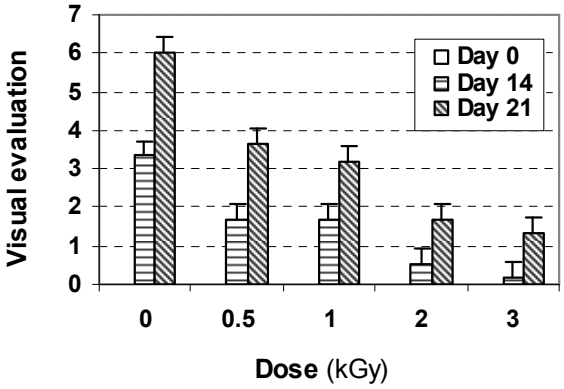

Figure 4. *Visual assessment of sea bass (Dicentrarchus labrax* L.*), irradiated by gamma rays at doses 0, 0.5, 1, 2 or 3 kGy and stored during 21 days at 1°C. Visual assessment was rated according to scale 0, 9. 9 indicated fish completely altered. Values are expressed as means ± SD for n= 3.*

For all measured deterioration parameters, interaction "dose x storage" is significant ($P < 0.01$), what means that the behaviour of fish samples during storage varies according to the dose of irradiation.

3.2. Effect of Irradiation on Membrane Lipids Degradation

PL, FS and FFA of sea bass muscles were measured during storage to verify if changes of TVB-N and TMA contents, pH, and external aspect of fish were associated to an alteration in membrane lipids composition and if they were influenced by irradiation.

Total PL content of sea bass muscles declined significantly during storage at 1°C for all treatments ($P < 0.001$; Fig. 5). However, this diminution varied according

to the dose of irradiation. Indeed, the rate of decline in PL level was less for irradiated samples than in the control principally for doses 1, 2 and 3 kGy.

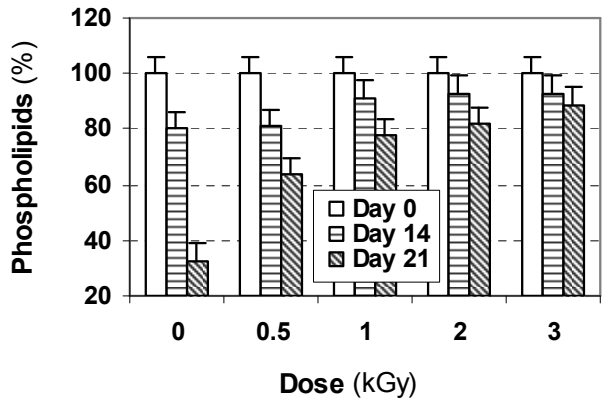

Figure 5. *Change with time of PL content of sea bass (Dicentrarchus labrax* L.*) muscles, irradiated by gamma rays at doses 0, 0.5, 1, 2 or 3 kGy and stored during 21 days at 1°C. Values are expressed as means ± SD for n= 3.*

The FS content showed no significant change under any of the treatments during storage of sea bass ($P > 0.05$). The loss of PL from the membrane during storage was reflected with a shift in the ratio of FS to PL ($P < 0.001$; Table 1). The ratio, which increased significantly for the control was closely correlated with the increase of TVB-N and TMA contents, and pH, and loss of external aspect (0.92, 0.93, 0.92 and 0.93, respectively).

Table 1. *Change with time in the ratio of FS to PL (μg/μg) to sea bass (Dicentrarchus labrax* L.*) muscles, irradiated by gamma rays at doses 0, 0.5, 1, 2 or 3 kGy and stored during 21 days at 1°C. Values are expressed as means ± SD for n= 3.*

Dose (kGy)	Days after storage		
	0	14	21
	FS/PL (μg/μg)		
0	0.13 ± 0.02	0.32 ± 0.06	0.47 ± 0.05
1		0.21 ± 0.02	0.33 ± 0.07
1.5		0.18 ± 0.05	0.28 ± 0.03
2		0.19 ± 0.03	0.26 ± 0.04
3		0.22 ± 0.02	0.31 ± 0.03

Table 2 shows the FA composition of PL and FFA fractions. PL are rich in linoleic acid (18:2), linolenic acid (18:3), eicosapentanoic acid (20:5) and docosahexaenoic acid (22:6), and their ratio of PUFA to saturated acids (mol%), 2.89, was greater than that of the FFA, 1.07.

Loss of PUFA from both fractions during storage was reflected by a decrease in the ration of PUFA to saturated FA (Tables 3 and 4). Nevertheless, the decrease was less important for irradiated samples than in the control ($P < 0.001$).

Table 2. *FA composition and ratio of PUFA to saturated FA (mol%) of the PL and FFA fractions of sea bass (Dicentrarchus labrax* L.*). Values are expressed as means ± SD for n= 3.*

FA	Fractions	
	PL	FFA
14:0	1.17 ± 0.3	4.76 ± 0.7
16:0	9.39 ± 0.5	18.94 ± 1.2
16:1	2.25 ± 0.2	11.10 ± 0.9
18:0	8.11 ± 0.4	8.89 ± 0.7
18:1	7.56 ± 0.4	27.32 ± 1.6
18:2	18.74 ± 1.3	7.77 ± 0.5
18:3	11.17 ± 0.9	3.66 ± 0.4
20:0	2.81 ± 0.2	2.03 ± 0.3
20:1	3.08 ± 0.5	2.24 ± 0.2
20:5	11.83 ± 0.4	1.33 ± 0.2
22:0	2.16 ± 0.1	3.01 ± 0.3
22:1	6.29 ± 0.3	1.01 ± 0.3
22:6	13.65 ± 0.8	6.30 ± 0.4
24:0	1.77 ± 0.2	1.60 ± 0.3
PUFA/S	2.89 ± 0.4	1.07 ± 0.3

Table 3. *Change with time in the ratio of PUFA to saturated FA (mol%) to the PL fraction of sea bass (Dicentrarchus labrax* L.*) muscles, irradiated by gamma rays at doses 0, 0.5, 1, 2 or 3 kGy and stored during 21 days at 1°C. Values are expressed as means ± SD for n= 3.*

Dose (kGy)	Days after storage		
	0	14	21
	PUFA/S		
0	2.90 ± 0.36	1.77 ± 0.21	0.61 ± 0.19
0.5		2.34 ± 0.17	1.89 ± 0.09
1		2.48 ± 0.16	2.02 ± 0.11
2		2.47 ± 0.23	2.08 ± 0.07
3		2.45 ± 0.20	1.85 ± 0.13

Table 4. *Change with time in the ratio of PUFA to saturated FA (mol%) to the FFA fraction of sea bass (Dicentrarchus labrax* L.*) muscles, irradiated by gamma rays at doses 0, 0.5, 1, 2 or 3 kGy and stored during 21 days at 1°C. Values are expressed as means ± SD for n= 3.*

Dose (kGy)	Days after storage		
	0	14	21
	PUFA/S		
0	1.07 ± 0.15	0.76 ± 0.09	0.49 ± 0.11
0.5		0.89 ± 0.07	0.77 ± 0.09
1		0.98 ± 0.11	0.91 ± 0.10
2		0.94 ± 0.08	0.95 ± 0.10
3		0.89 ± 0.20	0.87 ± 0.12

3.3. Effect of Irradiation on the Microorganisms Destruction

Table 5 shows the TVC of control and irradiated sea bass during storage at 1°C. The initial quality of fish used in this study was good, as indicated by a low initial value of TVC

(2.62 cfu/g) before fish were subjected to the different treatments. The irradiation just after treatment reduced significantly the level of microorganisms at 0.5, 1 and 2 kGy, and eliminated them totally at 3 kGy. Microbial counts of muscles tissue of all treatments increased significantly throughout the storage period but more quickly in untreated sea bass. Significant differences were observed also amongst the different irradiation treatments ($P < 0.001$).

Table 5. *Change of total viable counts (TVC) of sea bass (Dicentrarchus labrax* L.*) muscles during storage at 1°C after irradiation by gamma rays at doses 0, 0.5, 1, 2 or 3 kGy. Values are expressed as means ± SD for n= 6.*

Dose (kGy)	Days after storage		
	0	14	21
	TVC (Log CFU/g)		
0	2.64 ± 0.11	5.72 ± 0.09	8.43 ± 0.07
0.5	2.01 ± 0.13	4.12 ± 0.19	6.43 ± 0.12
1	1.35 ± 0.07	3.82 ± 0.14	4.32 ± 0.04
2	0.16 ± 0.01	1.92 ± 0.09	4.13 ± 0.08
3	00	0.78 ± 0.11	1.56 ± 0.07

4. Discussion

Sea bass is one of the more appreciated marine species farmed in many Mediterranean countries to meet the increasing demands on the part of consumers for fresh fish. Due to the perishable nature of fish and sea foods, this fish is also prone to spoilage during transportation to markets away from the place of catch. The beneficial effect of irradiation on the preservation of the quality and the freshness of aqua-acculturated sea bass and the control of the development of microorganisms has been demonstrated [11, 28]. However, to our knowledge there is no study which showed the gamma irradiation effect on the marine sea bass membrane lipids protection from degradation, which plays always an important role in the maintenance of tissue integrity of fishery products [15, 17]. Our study has shown that the irradiation slowed the degradation of membrane lipids and consequently prolongs the sea bass storage which preserved an interesting nutritional qualities and external aspect. Indeed characteristic changes of fish alteration such as the increase of TVB-N and TMA contents, which are considered as a valuable tool in the evaluation of fish quality because of their rapid accumulation in muscle under refrigerated conditions [29], and pH and the loss of external aspect, were observed during sea bass storage at 1°C. Our study shows that the irradiation influenced these changes which have been reflected by slowing of sea bass quality deterioration during storage. Such observations were reported on the cod fish, on the sardine and on aqua-cultured sea bass [11, 30, 31]. A characteristic feature of post-mortem fish muscle alteration is membrane deterioration due to lipid enzyme-degradation and ensuing destabilisation of the bilayer. Protection of membranes integrity from degradation implies a best polar head interaction of adjacent PL and consequently a more important control of functioning of enzymes associated [14, 15]. Our results indicated that deterioration of sea bass muscles during storage was delayed by irradiation such that reflected by the slowing of TVB-N and TMA production, increase of pH and the alteration of external aspect. Implication of membrane lipids degradation during storage was indicated by several markers of lipids degradation during storage such as reduce of PL content, larger ratio of FS to PL, increase in the level of PL degradation products FFA, and losses of PUFA from PL and FFA. The levels of these markers of membrane lipid degradation changed in parallel with the increase of TVB-N and TMA contents, and pH, and the alteration of the external aspect, common markers of sea products deterioration. This shows the important role of lipids membrane in the process of sea bass alteration. The lipids membrane implication in the process of degradation was reported by De Koning [14], and Olley and Lovern [12]. Protection of sea bass membrane lipids from degradation by irradiation is explained by the slowing of these changes and consequently by the reduction of its alteration. Effect of irradiation on some sea products preservation was observed on the shrimp by Paradis and Adambounou [8], on the tilapia by Al-Khantani *et al.* [32], on the carp by Szulk *et al.* [6] and on the mackerel by Lakshmanan *et al.* [13]. Despite this obviousness, the direct effect of irradiation in the preservation of membrane integrity was not demonstrated. Our study shows also that the irradiation slowed the development of the microorganisms which is considered among limiting factors of marine product storage [33]. In fact, their presence on the product is always accompanied notably by the loss of quality and the consumption of reserves [8]. PL deterioration in invaded fish easily takes places and limits the shelf-life of marine products during storage [15]. Hydrolysis, induced by phospholipases produced either by the sea products or the microorganisms, produces free fatty acids that undergo further oxidation to produce low-molecular weight compounds that are partially responsible for the rancid off-flavour and taste of fish products [15, 23, 34, 35]. Thus, the beneficial effect of the irradiation on the membrane lipids preservation observed in our study could be partially attributable to its effect on the micro-organisms destruction. In fact, the level of preserved PL during storage of sea bass was always correlated ($r= 0.89$; $P < 0.01$) to the presence of microorganisms on the muscle tissue. Then, dose 3 kGy, where the destruction of the micro-organisms was more important, the effect of the irradiation on the membrane lipids preservation was most interesting.

In conclusion, this study brings evidence to the importance of irradiation in the slowing of deterioration of sea bass during storage. Preservation of its qualities of the deterioration implies probably a protection of membrane lipids from enzymatic degradation of phospholipids which seemed to be partially reliable to the micro-organisms charge of muscle tissue.

Abbreviations

FFA, free fatty acid; FS, free sterol; PL, phospholipids; PUFA, polyunsaturated fatty; TMA, trimethylamine; TVB-N, total basic volatile nitrogen; TVC, total viable counts.

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