Effects of Oxygen-free Water on Preservation of Threadfin Bream (*Nemipterus hexodon*) & Kuruma Prawn (*Penaeus japonicas*)

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Abstract— Effects of oxygen-free water on the preservation of threadfin bream was studied. The threadfin bream (*Nemipterus hexodon*) and Kuruma prawn (*Penaeus japonicas*) are of significant economic values in Thailand. To survey applicability of fine bubble (FB) technology to Thai fishes, 2 kinds of water and ice were tested at Mae Klong Fishery Cooperative LTD. (Mae Klong fish market) in Thailand; 1) oxygen free water (OFW), 2) normal tap water (NTW) and 3) ice made of tap water. After 12 days, sensory preference scores of the threadfin bream were evaluated for three treatments. The results revealed the positive effects in case of OFW and ice on physical qualities and sensory preferences of threadfin bream between Day 4 and Day 8. In addition, Kuruma prawn preserved in OFW was found excellent in freshness while prawn in NTW showed drastic color change of its head part and terrible smell. Consequently, OFW is found significantly effective in preserving the freshness of threadfin bream and Kuruma prawn.

Keywords—Oxygen-free bubbles water, preservation, Threadfin bream, Kuruma prawn

I. Introduction

Seafoods are one of major economic natural products and favorite diets in Thailand [1]. However, in their preservation, chemicals such as formaldehyde and formalin are generally being used as preservatives for tissues in Thailand due to quick deterioration of seafoods under tropical high temperature. This is simply because of no simple and safe alternatives, so far.

Many fish sellers in Thailand at present spray or dip fishes into formalin-treated water to keep seafood products look fresh for longer time, but endangers public health very much [2].

In 2011, the National Toxicology Program, an interagency program of the Department of Health and Human Services, USA named formaldehyde as a known human carcinogen in its *12th Report on Carcinogens* [3].

Formaldehyde (HCHO) presence in seafoods was often found in fresh markets, flea markets in the areas located mostly far away from the ocean [4]. Thus, fish preservation, or long shelf life, is a great concern in tropical countries like in Thailand. One example of formalin test made for shrimps and squids in Thailand is shown in Fig.1, using a pack test method.

Fine bubbles (FB) technology, bubbles consisting of micro bubbles (1μm<MB<100μm dia.), and ultra-fine bubbles (UFB<1μm dia.), is now rapidly emerging as an innovative technology in various fields [5].

One of such application is Oxygen-free water (OFW) for fish preservation [6], which is now being used in Japanese fish markets [7]. In this study, since fish species and climate of Thailand are vastly different from Japan, the applicability of this method to Thailand is investigated.

The mechanism in preservation of fish freshness is as follows; since fish degradation in water is caused by aerobic bacteria which decompose fish protein using dissolved oxygen in the water, oxygen-free water can suppress such bacteria's activity to great extent, leading to long preservation of fish freshness. The biggest merit of this method is totally safe without any chemicals.

This OFW can be easily made by using N_2-FB injection into tap water, thereby rising macro N_2 bubbles take out dissolved oxygen out of water surface, which penetrates into the bubble according to Henry law. Usually DO about 8 mg/L in normal tap water can be reduced down to 0.1～1 mg/L by this method, which is sufficient enough in suppressing bacteria activity.

For producing N_2-FB, a very less-expensive, yet high performance KVM01 FB generator was developed for basic research at RMUTL based on pressurized dissolved gas method (Fig.2). KVM-01 can produce not only microbubbles (MB) of several tens of thousands/mL, but also ultra-fine bubbles (UFB) as high as 10^{11} bubbles/mL, evaluated by a laser scattering particle analyzer, HORIBA LA-960A at RMUTL.

Actually, Oxygen ultra-fine bubbles were measured, and found to stay longer, as long as one moth, under suitable conditions [5]. It was also proved that free radicals are generated during the collapse of microbubbles, which can kill bacteria very strongly [8, 9].

Fig.1. Formaldehyde pack test results for shrimps and squids from fish market in the northern Thailand. From left, shrimps, squids, 2 ppm HCHO, and original HCHO.

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Fig.2. KVM01, flow rate of 1 L/min based on pressurized dissolved gas method developed by RMUTL.

On the other hand for OFW, it is necessary to keep OFW free from air contact to prevent oxygen re-dissolution into water.

In this study, we have examined the effects of Oxygen-free water on Thai's marine fishes, and compared with traditional methods that use normal water and ice.

II. METHODOLOGY

A. Fish Sources

Threadfin bream (*Nemipterus hexodon*) and Kuruma prawns (*Penaeus japonicus*) were supplied from the Mae Klong Fishery Cooperation LTD., (Mae Klong fish market), Samut Songkram Province, Thailand. Threadfin breams were approximately of 200 g in weight and tested in each treatments in plastic bags for 12 days in the iced storage box in the room at normal temperature, whereas Kuruma prawns of approximately 15 cm in length were preserved both in OFW and normal tap water in plastic bags in the refrigerator for 8 days for comparison.

B. Experimental Design

The threadfin bream experiments were made using a completely randomized design (CRD) comprising 3 treatments: (1) oxygen free water (OFW); DO = 1 mg/L, (2) normal tap water (NTW); DO = 7 mg/L, and (3) ice made of normal tap water.

Sixteen fishes were individually packed in polyethylene bags (PE), and then divided into four equal groups, each for 4 times' sensor tests in 12 days. Fish were soaked in each water type and kept in the iced storage box and left in the room at normal temperature. Ice in storage box was packed twice of fish weight, and refilled every day with the lost amount of ice [10].

Evaluation of the physical quality change was made every 4 days during total period of 12 days. The observation of physical parameters with respect to eye color, gill, skin, odor and texture were made on the 1st and 12th day, and compared.

In addition, pre-experimental design was used for Kuruma prawn and preserved in OFW for 8 days, and then compared with normal tap water. In this study, the 1st sensory analyses were made by veteran fish experts at the Mae Klong fish market and the 2nd test were evaluated by trained panelists at RMUTT (Rajamangala University of Technology Thanyaburi, Pathum Thani, Thailand), of threadfin beams for color (eyes and gill), skin, odor and texture, using sensory scores, where 1 = least, 2 = less, 3 = moderate (i.e. neither good nor less), 4 = good, and 5 = excellent, respectively [11,12].

Oxygen-free water was made at RMUTT relatively near Mae Klong fish market by using KVM-01. Prior to production, once-through cleaning of whole generator channels was conducted by prepared original water for 20 minutes, with flow rate of 1 L/min [13]. Then nitrogen FB was injected by the FB generator to 10 L water in the plastic bottle for 15 min. with nitrogen gas flow rate of 0.05 L/m, resulting in DO=1 mg/L water.

Produced OFW was then packed into a 10L plastic hard bottle, and brought to the Mae Klong fish market about 3 hours-drive apart. Regarding OFW, however, in the first experiment made in November 2017, the water was first used about 1 L to prawn test and brought back to RMUTT, and some days later, this remaining 9 L water was used for preservation of threadfin breams at the fish market.

As will be discussed later more in details, it turned out, that the water used in the 1st experiment for Threadfin breams was ***no more*** oxygen-free water, just normal water containing DO = 7 mg/L or so, due to the contact with 1 L volume air inside the upper space of the bottle, where 1 L OFW occupied before prawn test.

Therefore, in the 2nd experiment in March, 2018, the same 10 L OFW with DO = 1 mg/L was prepared. OFW was entirely and immediately used for fish preservation without any delay, to keep OFW as real OFW.

III. RESULTS

A. Prawn preservation by oxygen-free water

At the fish market, two prawns were packed in the sealed plastic bags, one in oxygen-free water (OFW), and the other in normal tap water (NTW), and kept in the refrigerator for 8 days at RMUTT.

The results are shown in Fig.3, where the prawn preserved in OFW was found excellent as fresh as was brought to the market, whereas the prawn in NTW showed drastic color change of its head part and smelled terrible, i.e., totally degraded.

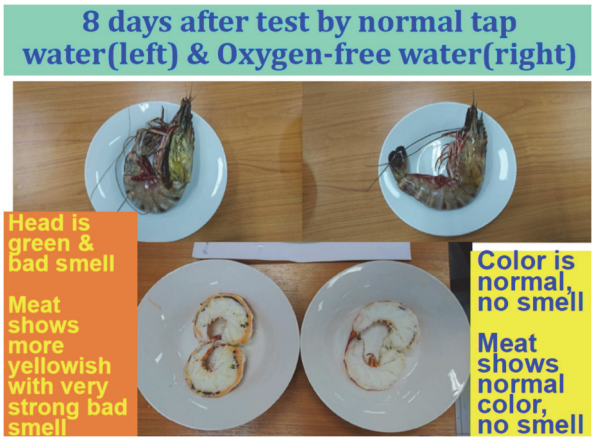

Fig.3. Two prawns preserved in tap water (left) and oxygen-free water (right) 8 days after treatment.

B. *Threadfin bream preservation by 3 kinds of treatments*

Several days later after Kuruma prawn test was made, the 1st preliminary preservation test for threadfin breams were made by Mae Klong fish market staffs, by using remaining 9 L "OFW"(actually no more oxygen-free water), NTW and ice, and results of sensory preference scores are shown in table I.

The results on Day 4 showed that the greatest score of overall acceptability was ice, followed by NTW and "OFW", respectively, whereas the results on Day 8 indicated that "OFW" is similar to ice (score = 4). The overall results in the 1st preservation test had the high score in sequence, ice, NTW, and "OFW" respectively, when considered in each parameter shown in Table I.

Likewise, the 2nd preservation test (Table II) showed the different scores of overall acceptability, in Day 4, ice showed highest score, followed by NTW and OFW, respectively. By comparison, the OFW results of sensory preference score of eyes and skin showed the better score when compared on the 1st preservation results.

At the end of experiments, the sensory preference score of OFW including odor and texture showed to improve when compared with the 1st test. Overall, ice exhibited high score, followed by OFW, and NTW, respectively. Both of two experimental results exhibited that ice made of NTW could be most efficient for fish preservation.

From these experiments, OFW showed the positive effects on the meat quality and sensory preference of Threadfin bream between Day 4 and Day 8. In addition, the overall results indicated that OFW could preserve the freshness of threadfin bream until Day 8.

IV. Discussion

The reasons for fish quality deterioration leading to spoilage need to be determined carefully. The time of pre-rigor mortis and rigor mortis varies according to species. It depends also on many factors, such as temperature, handling, size, physical condition, bio-chemical reactions and microorganism activities of the fishes [2, 14].

In the Food and Agriculture Organization (FAO)'s comprehensive reviews on utilizing ice in fish preservation, when ice is utilized for reducing temperature to about 0 ºC, the growth of spoilage and pathogenic micro-organisms are reduced [15], as well as the rate of enzymatic reactions, in particular, those linked to early rigor mortis period. As was mentioned, it is most likely that after 1 L use of OFW from the 10 L OFW sealed bottle, air was introduced and stayed in 1 L space of the bottle.

In case just after 1 L air enters the 10 L plastic bottle containing 9L OFW with DO = 1 mg/L, then, total oxygen in the 9L water = 1 mg/L×9 L = 9 mg, whereas total oxygen in 1 L air = 0.2×32 g/mol×(1/22.4) = 286 mg showing overwhelming large quantity of oxygen in the air space, leading to prompt equilibrium state of dissolved oxygen in the water.

Therefore, the 9 L remaining initial oxygen-free water is expected to return quickly to normal water having approx. DO = 7 mg/L, and as aforementioned that this "OFW" is no more oxygen-free water, but normal tap water, in terms of dissolved oxygen level.

TABLE I
SENSORY PREFERENCE SCORES OF THREADFIN BREAM FOR 3 TREATMENTS; "OFW" PRODUCED BY KVM-01 DURING NOVEMBER 11-22, 2017 (1ST TEST).

Parameters	Treatment	Start	Day4	Day8	Day12
Eyes	1. OFW	5	4	4	3
	2. NTW	5	4	4	3
	3. Ice	5	5	4	4
Gill	1. OFW	5	5	4	4
	2. NTW	5	5	4	4
	3. Ice	5	5	4	4
Skin	1. OFW	5	4	4	3
	2. NTW	5	4	4	3
	3. Ice	5	5	4	4
Odor	1. OFW	5	4	4	3
	2. NTW	5	5	3	3
	3. Ice	5	5	4	4
Texture	1. OFW	5	4	4	3
	2. NTW	5	5	4	4
	3. Ice	5	5	4	4
Overall acceptability	**1. OFW**	**5**	**4.2**	**4**	**3.2**
	2. NTW	**5**	**4.6**	**3.8**	**3.4**
	3. Ice	**5**	**5**	**4**	**4**

Remarks: 1 = least, 2 = less, 3 = moderate (i.e. neither good nor less), 4 = good, and 5 = excellent

TABLE II
SENSORY PREFERENCE SCORES OF THREADFIN BREAM FOR THE 3 TREATMENTS; "OFW" PRODUCED BY KVM-10 DURING MARCH 2-14, 2018 (2ND TEST).

Parameters	Treatment	Start	Day4	Day8	Day12
Eyes	1. OFW	5	5	4	3
	2. NTW	5	5	3	3
	3. Ice	5	5	4	4
Gill	1. OFW	5	5	4	4
	2. NTW	5	5	3	3
	3. Ice	5	5	4	4
Skin	1. OFW	5	5	4	3
	2. NTW	5	4	3	3
	3. Ice	5	5	4	4
Odor	1. OFW	5	4	4	4
	2. NTW	5	5	3	2
	3. Ice	5	5	4	4
Texture	1. OFW	5	4	4	4
	2. NTW	5	5	3	3
	3. Ice	5	5	4	4
Overall acceptability	**1. OFW**	**5**	**4.2**	**4**	**3.6**
	2. NTW	**5**	**4.6**	**3**	**2.8**
	3. Ice	**5**	**5**	**4**	**4**

Remarks: 1 = least, 2 = less, 3 = moderate (i.e. neither good nor less), 4 = good, and 5 = excellent

In the actual application in the fish market, gas-barrier flexible plastic bags which contain OFW should be used for fish preservation to avoid air invasion into the bags. However, the results in the 2nd preservation test indicated that OFW had good score and keep freshness longer than the 1st time due to immediate use of OFW for fish preservation the OFW in the 2nd experiment. Although, oxygen is necessary for the growth of aerobic bacteria, however, nitrogen gas flushing can be deleterious to bacteria [16, 17]. Thus, in our experiments, nitrogen bubbling in a form of ultra-fine bubbles could penetrate into fish meat through skin and visceral, and prevented oxidation of oil and fat content and the activity and growth of aerobic bacteria as well. Under such condition freshness is kept at least 8 days with good taste. [18]

V. Conclusion

The results revealed that ice is still the most suitable way for fish preservation. However, the oxygen-free water also exhibited the positive effects on the physical quality and sensory preference of threadfin bream giving shelf life for 8 days. In addition, the preservation of Kuruma prawn in oxygen-free water was found excellently fresh. The experiments could be confirmed the good applicability of fine bubble technology as an alternative way for preservation of marine fish products.

Acknowledgment

The authors would like to express sincerest gratitude to Mr. Anuthep Wongvarit, Mr. Suradej Tunyuchok, Mr. Chinchai atirayakorn, Mr. Adisorn Dechboon and all the staff members of the Mae Klong Fishery Cooporation LTD., Thailand for their invaluable advices and very careful experiments on fish preservation.

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