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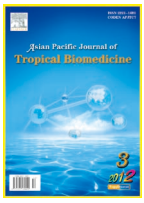

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The Effect of Various Methods of Defrosting on Microbial Contamination of Frozen Banana Shrimp (*Penaeus merguiensis*)

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ABSTRACT

Objective: Background and aim: The most common and the best way of preventing microbial decay of marine foods is using freezing technology and the cycles and methods of defrosting have considerable effect on microbial changes of frozen shrimp. Shrimp is one of the marine foods that due to high active water (aw) and neutral PH and autolytic enzymes have high decay. Thus, in this study the effect of various methods of defrosts on microbial contamination of shrimp was investigated. **Methods:** This study was an empirical design on *Penaeus merguiensis*. The shrimps were divided into three groups including 1- peeled and headless (PUD), 2- Complete, 3- Headless (with skin) being frosted and defrosted in three cycles. Each group of shrimp was classified in terms of the type of defrosting method in three groups as 1- Microwave, 2- Refrigerator, 3- Water and were investigated in 3 cycles with the interval of 4 days. In this investigation, the total bacteria, Psychrophil bacteria, coliforms and Staphylococcus aureus were counted in private culture mediums. For data analysis, repeated measure Anova was used. **Results:** All the bacteria including Psychrophil bacteria, coliforms and Staphylococcus aureus during the cycles had significant reduction process and this reduction showed significant reduction in complete shrimp and defrosting with refrigerator compared to other groups ($P<0.05$). **Conclusions:** According to the results, complete shrimp was the best kind of shrimp in terms of microbial load. Thus, avoiding temperature changes during transportation and avoiding unduly defrosts in maintaining the quality of the frozen shrimp is proposed.

1. Introduction

The present study aims to evaluate the effects of freezing and thawing methods on the bacteriological quality of Banana Shrimp (1). The most important aim of keeping food is the delay of the growth of decaying microbes and reducing them. Marine foods due to high active water and neutral PH and the presence of autolytic enzymes have high decaya [2].. The best way to prevent the decay is freezing technology because storing marine foods at suitable temperature keeps their quality [3]. Different freezing and thawing techniques have been used for various foods [4]. Shrimp is a highly decayed marine food. The main factors facilitating the decay process of shrimp are microbial activities and enzyme reactions [5]. Furthermore, lipid oxidation can lead to off-flavor development in shrimp (6). The quality of the shrimp food depends upon various factors including the freezing speed and defrosting, the temperature of storing location, temperature changes, freezing and defrosting frequencies, transportation and consumption method. Defrosting during an unsuitable transportation and frequent packing have adverse impacts on the quality of shrimp and enhance the decay process [7]. Some factors including muscle cells destruction and release of mitochondrial enzymes from sarcoplasm, increased lipoxidase activity and even the type of frozen shrimp in defrosting process can lead to physical, chemical and microbiological changes in the frozen food [8,,,F]. Therefore, assessment of effects of freezing and defrosting cycles on microbial changes of frozen shrimp is of great importance [2]. Jiaskaran et al. (2006) showed that the number of total bacteria, coliform, Psychrophil and Lactic acid bacteria in the form of frozen shrimp in Indian white shrimp during storing time was reduced considerably[2]. Lakshmisha (2008) showed a significant decreasing trend in total plate count

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in commercial plate and air blast freezers in mackerel [1]. Muela (2010) revealed that storing time of meat was effective on the amount of bacteria producing lactic acid. But these scientists didn't observe an exact relationship between the change in the number of lactic acid bacteria and frosting and defrosting cycles of meat [10]. Neither freezing method nor frozen storage duration up to 6 months influenced significantly the instrumental quality of lamb [10]. Previous studies demonstrated that freezing temperatures are the most crucial factor determining the quality of food after thawing [11, 9]

The shrimp can be kept physically by various methods including 1- peeled and headless (PUD), 2- Complete, 3- Headless (with skin) that is different depending upon the number of existing bacteria in shrimp. For example, the headless type has less microbial hazard than on-head type because the head of the shrimp is including 75% of total bacteria [5]. In a study carried out by Bayler et al. (1973), it was defined that the shrimps being stored by their head had less total bacteria population and more dry substance and they were better than head less one in terms of organoliptic [12]. In another study performed by Zapatka and Bartolomo (1973), it was shown that complete shrimp had less bacteria compared to PUD and headless shrimps [13].

There are various defrosting methods including air, water and microwave defrosting. There is no index to define the best method [14]. In air defrost, food packages less than 10cm are defrosted easily at room temperature but this method is not recommended for thick packages. The advantage of this method is its requirement to less equipment [14]. In water defrosting, the food is placed at room temperature in water. In this method, due to higher thermal transfer coefficient, defrost speed is higher. The problem of this method is putting the food in water [14]. Microwave defrosting is created by the direct effect of electromagnetic waves to the product without using electrode. Microwave is a rapid method. But one of the disadvantages of this method is that some part of the frozen production is cooked while other part is at frozen state [3]. In a study performed by Bonsomerji et al. (2007), it was shown that microwave method had the highest damage and refrigerator method had the least damage among different kinds of defrosting the shrimp [15].

2. Materials and methods

This study was empirical being performed on the shrimps obtained from Persian Gulf. The sample shrimps were selected randomly. They were transferred with ice to a lab in Qeshm city in a fish production factory of Qeshm Soza. Microbial factors including 1-The total count of microbes, 2- The count of Psychrophil, 3- The count of bacteria producing lactic acid, 4- The count of coliforms, 5- The count of Staphylococus Aureus were measured.

Other samples were divided into three groups 1- PUD, complete, 3- Headless (with skin) were frozen in polyethylene of 200g at -40 . Then, the samples were transferred to Kerman vet school and were kept at -18 in freezer. After four days of fishing, defrosting was done by three methods of 1- Microwave, 2- Refrigerator, 3- Sterile distilled water such that three various groups of shrimps were defrosted every four days by three defrosting methods. Of each group, 10 g was taken for the test and the rest of the shrimps were frozen again at 18 and finally for each group of shrimp, three cycles of freezing and defrosting were registered.

1. Defrosting by microwave: The samples were placed in a dish and were adjusted in microwave (made in LG company of Korea, model MC-8084WR101-230V~50HZ) on automatic defrosting of marine food and the time was set based on the weight of the samples automatically and the samples were defrosted without being cooked.

2. Defrosting by water: The samples were placed in sterile water with the temperature of 22 and after 30min, the temperature of the samples reached 22degree and defrosting was complete.

3.Defrosting by refrigerator: The samples were placed in a sterile dish in refrigerator at temperature 4 to 5 degree and after 5hours, the samples were defrosted.

These three methods of defrosting were repeated in each three groups with the interval of 4 days in three cycles.

2.1. Doing the experiment

10g of the samples were mixed with 90cc sterile physiology serum by bag mixer model VW made in France and a homogenized solution was obtained. 1 cc of it was poured in a tube consisting of 9 cc sterile physiology serum and the dilution of 10-6 was prepared. Then, each of the dilutions was taken to the culture medium to measure the microbial factors. The count of the total number of bacteria was done in culture medium TSA (made in High media of India) at 37 for 24hours, the count of Psychrophil bacteria in TSA culture medium at 35 for 7 days, the count of bacteria producing lactic acid in Lactobacillus MRS Agar culture medium (made in high media of India) at room temperature for 24 hours and the count of Staphylococcus aureus was done in Baird Parker medium (made in Merck company of Germany) at 37 for 48 hours.

2.2. Data analysis

For data analysis, variance analysis with repeated measure ANOVA was applied in which the type of shrimp, the method to take out from the freezer and time were used as independent variables and different kinds of bacteria were dependent variables. SPSS software was used for statistical analysis.

3. Results

The results in three groups of shrimps (complete and headless with skin and PUD) in three methods of defrosting by microwave, water and refrigerator in three consecutive cycles with the interval of 4 days are as follow:

The results of total bacteria in each group by three methods of freezing showed that the number of total bacteria during the cycles had significant reduction and this reduction showed significant different considering the type of shrimp and they type of freezing method ($P<0.05$) (Table 1).

Regarding Staphylococcus aureus, Coliform and Psychrophil, the number of the bacteria was reduced in three cycles and there was a significant different among various shrimp groups by different defrosting methods ($P<0.05$) (Tables 2, 3, 4).

Regarding the bacteria producing Lactic acid based on the type of shrimp and the type of defrosting method, there was no statistical different among various groups (Table 5).

Among various groups of shrimps, complete shrimp showed less reduction in terms of the number of microorganisms compared to two other shrimps, PUD and headless one

(P<0.05). Among three kinds of defrosting methods, the number of bacteria in defrosting by refrigerator had the highest reduction and in microwave defrosting, had the least reduction (P<0.05).

4. Discussion

In this study, the total bacteria were investigated in three including 1- peeled and headless (PUD), 2- Complete, 3- Headless (with skin) in three cycles of defrosting by microwave, water and refrigerator in three consecutive cycles with the interval of 4 days. By the increase of the number of cycles in three methods of defrosting, the number of bacteria was reduced and this reduction showed significant different based on the type of shrimp and the type of defrosting method. Regarding Staphylococcus aureus , Coliform and Psychrophil bacteria, the number of the bacteria was reduced in three cycles and there was a significant different among various shrimp groups by various defrosting methods. However, regarding lactic acid bacteria, there was no significant relationship between the type of shrimp and defrosting method. Based on the results of the study, freezing and defrosting method affect the number and the type of shrimp bacteria and its texture and it is approved in other papers. For example, Jiaskaran et al. (2006) showed that they number of total bacteria, coliform, Psychrophil and lactic acid bacteria were reduced considerably during

Table 1

The average number of total bacteria in Total test in three methods of defrosts during three consecutive cycles in each group of shrimp. Values are mean (SD)

Cycle	Headless shrimp			Complete shrimp			PUD Shrimp		
	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator
1	16500 ±2121/32	17000	12500±3535/53	13000±1414/21	10500 ±707/1	1555.63±10900	14500±707/106	4242/64±14000	19700±15980/61
2	18000±11313/71	4101/21±5100	2450±1060/66	7800±2545/58	3650±2757/71	1060/66±2250	8950±636/39	6100±14142/13	4750±353/55
3	48000±36769/55	3500±2545/58	1250±353/55	5100±2687/005	1600±28274	1000±141/42	6550±1343/5	3800±1131/37	2750±2050/6

Table 2

The average number of bacteria in Staphylococcus aureus test in three methods of defrosts during three consecutive cycles in each group of shrimp. Values are mean (SD)

Cycle	Headless shrimp			Complete shrimp			PUD Shrimp		
	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator
1	95±7/07	75±21/21	45±7/07	35±7/07	20	10	60±14/14	45±7/07	35±7/07
2	55±7/07	45±7/07	30±14/14	10±14/14	0	0	30±14/14	25±7/07	15±7/07
3	15±7/07	15±7/07	10	0	0	0	5±7/07	5±7/07	0

Table 3

The average number of bacteria in coliform test in three methods of defrosts during three consecutive cycles in each group of shrimp. Values are mean (SD)

Cycle	Headless shrimp			Complete shrimp			PUD Shrimp		
	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator
1	2300±282/84	5850±494/97	1300±141/42	90±14/14	350±212/13	20±14/14	185±21/121	395±49/49	95±7/07
2	390±14/14	725±148/49	220±28/28	0	10±14/14	0	105±7/07	140±14/14	45±35/35
3	95±7/07	235±9192	55±49/49	0	0	0	22±3/53	41±12/72	0

Table 4

The average number of bacteria in Psychrophil test in three methods of defrosts during three consecutive cycles in each group of shrimp. Values are mean (SD)

Cycle	Headless shrimp			Complete shrimp			PUD Shrimp		
	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator
1	6150±494/97	4300±282/84	4100±424/26	2000±424/26	940±84/85	560±155/56	5100±282/84	3400±424/26	2100±282/84
2	4150±494/97	3350±1202/08	1650±212/13	800±212/13	500±141/42	215±91/92	1650±212/13	1150±212/13	1000
3	1950±353/55	1030±98/99	275±35/35	325±77/78	190±14/14	110±14/14	1100±141/42	750±212/13	240±28/28

Table 5

The average number of bacteria producing lactic acid test in three methods of defrosts during three consecutive cycles in each group of shrimp. Values are mean (SD)

Cycle	Headless shrimp			Complete shrimp			PUD Shrimp		
	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator	Microwave	Water	Refrigerator
1	12500±14849/24	1150±212/13	385±21/21	3600±141/42	2750±353/55	1050±70/71	1800±282/84	875±176/77	410±14/14
2	17±3/53	47±3/53	77±./53	620±28/28	630±42/42	310±1414	230±42/42	475±35/35	175±35/35
3	210±14/14	195/7/07	175±35/35	2100±141/42	2050±70/71	1100±141/42	600±141/42	575±35/35	675±35/35

storing as frozen [16]. The results of this study were in line with our study. Rejsan et al. (2006) in a study on chicken showed that the number of coliform bacteria didn't have special sensitivity to freezing process and they were not considerably reduced [17]. However, in our study the number of these bacteria had descending process in freezing and defrosting cycles and this contraction can be due to the difference in the type of meat and freezing temperature, etc. In a study performed by Ersoy et al. (2008) on eel, it was defined that the total number of bacteria and coliform were reduced in all kinds of defrosting by air, microwave, water and refrigerator. In this study, only one freezing and defrost cycle was d one and the best method for defrosting was defrost by water in terms of microbial aspects. In this study, the eels were placed in a poly ethylene cover and then they were placed in water and they were defrosted, while in the current study, the shrimps were placed in water after removing the cover [18].

In a study performed on complete and headless shrimps of Bayler et al. (1973), it was shown that the shrimps being stored with their heads had less total bacteria population and less dry substance and they were better than headless shrimp in terms of organoliptic aspects [12]. In another study done by Zapatka and Bartolomo (1973), it was shown that complete shrimp had less bacteria compared to PUD and headless shrimps [13]. In another study conducted by Edel et al (2012), they showed that the activities of psychrophilic, mesophilic, thermophilic and spore-forming bacteria were reduced after freezing and thawing processes [5]. Zhou et al (2010) showed although some of the food preservation techniques are efficient at inactivation of the micro-organisms responsible for food-borne diseases, they are not effective against spores [7].

Furthermore, findings of a study performed on the quality of Coleen Leygonie meat (2012), showed that neither freezing nor thawing can decrease the number of viable microbes in meat. However, during the freezing process, inactivation of microbes can efficiently terminate the microbial deterioration.

Unfortunately, they can recover themselves and repossess their activity during thawing. Since thawing process is rather slower and less uniform than freezing, certain areas of the meat will be exposed to more favorable temperature conditions for microbial growth. This is of particular concern when air thawing is employed [3].

In our study, complete shrimp was in better conditions compared to PUD and headless shrimps in terms of the number of microorganisms. This showed that taking the head of the shrimp out causes that the shrimp is exposed to its gastric system, people hands and all surfaces including dock, ice cubes, etc and the protective effect of the skin on the flesh of shrimp is removed. This shows that despite the public image, complete shrimp with its gastric system during freezing and defrosting is a good shrimp in terms of microbial load

5. Conclusion

According to the results, a complete shrimp is the best type of shrimp in terms of microbial load and as food is defrosted by various methods in the distance between the initial freezing and consumption in various stages including transportation, selling in the store and consumption, considerable quantity and quality changes are occurred during these freezing and defrosts. Increasing the frequency of freezing and defrost by any method and any shrimp make Considerable quantity and quality changes

Conflict of interest statement

We declare that we have no conflict of interest.

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