

Contamination of Bacteria in Chilled Fresh Tabtim Fish (hybrid tilapia *Oreochromis* sp.) Processing to Assess the Critical Point of Processing

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ABSTRACT

A quantitative analysis of the loads of total aerobic bacteria, *Escherichia coli*, Coliform and *Staphylococcus aureus* in operational units of chilled fresh Tabtim fish (hybrid tilapia *Oreochromis* sp.) production was collected from six steps of processing which were tested by 3M™ Petrifilm. The results of the total aerobic bacteria and *E.coli* indicated that the highest contamination step was the process that packed Tabtim fish in polyethylene bags after chilled fish at 4 °C for 7 days and that Tabtim fish was knocked which showed the level of contamination 4.4×10^4 , <10 CFU/cm² respectively. Therefore, total aerobic bacteria and *E. coli* results were not over the standard value in TACFS 7001-2004. Any way, the Coliform and *Staphylococcus aureus* indicated that the highest contamination step was the process which fish was wiped with sponge before being packed showed the level of contamination 6.6×10^3 and 3.4×10^2 CFU/cm², respectively. No report has known before about the Coliform and *Staphylococcus aureus* in Tabtim fish. The results would be used to assess the critical point of chilled fresh Tabtim fish which was contaminated with these pathogenic bacteria.

Key words: bacteria, 3M™ petrifilm, tabtim fish, critical point, processing

INTRODUCTION

Recently, the demand of Tabtim fish (tilapia) consumption has increased continuously because Tabtim fish is low price with high nutrition food. The whole fish and the fillet are admirable for consumers. As a result, it affects the trend of both domestic and export consumption. Moreover, the Tabtim fish has many outstanding advantages such as easy to culture, high growth rate, easy breeding, high fibrilla protein, good taste, white cotton meat like sea bass fish, high nutrition and having more omega-3 than other wild freshwater fishes and wild estuarine fishes. (Aquatic animal

research center Charoenpokphand, 1999) In addition, the Tabtim fish has no evidence epidemic problem. It is wide spread cultured in every parts of Thailand. Also, this kind of fishes can be cooked for many types of food with a good price.

However, one important problem that the importers concerned for importing Tabtim fish was the microbiological quality. It should be met with the standard each country sets. This standard was very important and each country will use it to make sure about the aquatic animal quality. (Sangjindawong, 1995). After the fish died, the bacteria from viscera could even get into the fish meat directly through the skin and alimentary

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canal. The great amount of bacteria at the skin of the fish at the beginning would cause the fish to be rotten more rapidly. Therefore, the management could control and reduce the initial number of microorganism in the process of preservation after catching and the process of chilling fresh fish maintain the freshness of fish. It also reduced the problems about the spread of cross-contamination. (Watanasinthu, 2000).

In this research, the amount of contaminated bacteria in the chilled fresh Tabtim fish process, focusing on the bacteria causing alimentary canal illnesses such as total count of aerobic bacteria, *Escherichia coli*, Coliform and *Staphylococcus aureus* were studied in order to assess the critical point processing.

MATERIALS AND METHOD

Fish samples preparation

The samples were collected from 6 steps (the step of knocking the Tabtim fish, the step of scaling, eviscerating and cutting-up the fish, the step of wiping the Tabtim fish with sponge before packing and the step of packing the Tabtim fish with polyethylene bags and chilled at 4°C in the refrigerator for 7 days) of chilled fresh Tabtim fish process. In each step, were collected to do triplicate tests for 3 types of 3M™ petrifilm (3M Thailand, 2005). The total tests of each collection were 216. The samples were collected from used water and ice in processing line, swabbing 100 cm² of fish's skin after knocking fish, scaling, eviscerating and cutting-up fish, wiping with sponge fish and fish packed in polyethylene bags and chilled at 4°C after being stored in the refrigerator for 7 days, respectively.

Enumeration methods

The enumeration on skin fish (swabbing 100 cm² for each sample) was analyzed by 3M™ petrifilm technique, (3M Thailand, 2005). The samples were collected at each step of processing were inoculated onto 3 types of 3M™ petrifilm which are Total Aerobic Count Plate, *E. coli* and

Coliform Count Plate, and Staph Express Count Plate. Then, all the samples were inoculated on to each petrifilm that were incubated the Petrifilm Aerobic Count Plate at 35°C for 48 hours (AOAC, 1994), Petrifilm *E.coli* and Coliform Count Plate at 35°C for 24 hours (AOAC, 2002) and Petrifilm Staph Express Count Plate at 35°C for 24 hours (AOAC, 2003). The results of colony counting per ml were followed in types of media which are showed the 25-250 CFU/ml of red colony spot after incubating for 48 hours in Total Aerobic Count Plate (Curiale *et. al.*, 1990), the 15-150 CFU/ml of only dark blue spots and has some gas bubble after incubating for 24 hours in *E. coli* (Gangar *et al.*, 1999), the 15-150 CFU/ml of both of red spots and dark blue spots that have some gas bubble after incubating for 24 hours in Coliform (Gangar *et al.*, 1999) and the 1-150 CFU/ml of colony of red-violet spots after incubating for 24 hours in *Staphylococcus aureus* (Horwitz and Latimer, 2005).

RESULTS AND DISCUSSIONS

The contamination of bacteria in chilled fresh Tabtim fish (hybrid tilapia *Oreochromis* sp.) causes from non-hygienic processing. The results of quantity analysis of Total Aerobic Bacteria, *Escherichia coli*, Coliform and *Staphylococcus aureus*, taken samples from chilled fresh Tabtim fish process and tested by 3M™ petrifilm from January to June, 2006 could be concluded in Table 1. The average of quantitative value of contamination of bacteria from chilled fresh Tabtim fish process from highest to lowest within total aerobic bacteria contaminating which was indicated as follows; the step of packing the Tabtim fish in polyethylene bags and chilled at 4°C in the refrigerator for 7 days, the step of wiping the Tabtim fish with sponge before packing, the step of scaling, eviscerating and cutting-up the fish and the step of knocking the Tabtim fish, respectively. The highest to the lowest steps of *E. coli* contaminating were indicated as follows; the step of knocking the Tabtim fish, the step of scaling,

Table1 Average of quantitative value of contamination bacteria on fish skin from chilled fresh Tabtim fish process from January to June 2006. (CFU/cm²).

Step	Total aerobic bacteria	<i>E. coli</i>	Coliform	<i>Staphylococcus aureus</i>
1	9.2×10^2	< 10	1.6×10	0.23×10
2	4.3×10^3	<10	7.4×10^2	1.2×10
3	2.1×10^4	<10	6.6×10^3	3.4×10^2
4	4.4×10^4	<10	0.4×10	<10

Remark: Step of fish processing were knocking fish (1); scaling, eviscerating and cutting-up fish (2); wiping the tabtim fish with sponge (3); packing in polyethylene bags and chilled at 4°C in the refrigerator for 7 days.

eviscerating and cutting-up the fish, the step of wiping the Tabtim fish with sponge before packing and the step of packing the Tabtim fish with polyethylene bags and chilled at 4°C in the refrigerator for 7 days, respectively. The highest to the lowest of coliform contaminating were indicated as follows; the step of wiping the Tabtim fish with sponge before packing, the step of scaling, eviscerating and cutting-up the Tabtim fish, the step of knocking the Tabtim fish and the step of packing the Tabtim fish in polyethylene bags and chilled at 4°C in the refrigerator for 7 days, respectively. Finally, the highest to the lowest of *Staphylococcus aureus* contaminating were indicated as follows; the step of wiping the Tabtim fish with sponge before packing, the step of scaling, eviscerating and cutting-up the Tabtim fish, the step of knocking Tabtim fish, and the step of packing the Tabtim fish with polyethylene bags and chilled at 4°C in the refrigerator for 7 days, respectively.

Thai Agricultural Commodity and Food Standard (TACFS) 7001-2004 is mention the critical point of Tilapia in case of bacteria contamination in meat of the fish at total viable bacteria count is not greater than 1×10^7 CFU/gram of meat or 5×10^5 CFU/gram of meat from 3 samples in 5 collected samples. That mean in this study, the total aerobic bacteria average amounts of bacteria contamination at the step of packing chilling fresh Tabtim fish in polyethylene bags and chilled at 4°C in the refrigerator for 7 days (4.4×10^4 CFU/cm²) and the step of wiping the Tabtim fish with sponge before packing (2.1×10^4 CFU/cm²) were less more than TACFS 7001-

2004. Because at the step of scaling, eviscerating and cutting-up the fish were found the total aerobic bacteria average amounts of bacteria contamination at 4.3×10^3 CFU/cm² contamination at the step of packing chilling fresh Tabtim fish in polyethylene bags and chilled at 4°C in the refrigerator for 7 days and the step of wiping the Tabtim fish with sponge before packing. Then, we should ask the processors to beware or to reduce the bacteria contamination at the step of scaling, eviscerating and cutting-up the fish. The highest average amounts of *E. Coli* is not detected (<10 CFU/cm²). But the average amounts of Coliform contaminating are 6.6×10^3 , 7.4×10^2 , 1.6×10 and 0.4×10 CFU/cm² at the step of wiping the Tabtim fish with sponge before packing, the step of scaling, eviscerating and cutting-up the Tabtim fish, the step of knocking the Tabtim fish and the step of packing the Tabtim fish in polyethylene bags and chilled at 4°C in the refrigerator for 7 days, respectively. That mean, the chilling process of fresh Tabtim fish was contaminated with some pathogenic food poisoning or causing the alimentary canal illnesses. While the average amounts of *Staphylococcus aureus* is also as 3.4×10^2 , 1.2×10 , 0.23×10 and <10 CFU/cm² at the step of wiping the Tabtim fish with sponge before packing, the step of scaling, eviscerating and cutting-up the Tabtim fish, the step of knocking Tabtim fish and the step of packing the Tabtim fish with polyethylene bags and chilled at 4°C in the refrigerator for 7 days, respectively. So the critical point of chilled fresh Tabtim fish was 4 points as same as the process because if the contamination with these pathogenic

bacteria is loaded, the shelf life of products will short expire and hazard to consumer. So we should control the load of contaminations of each processing by limiting the quantity of fish to be knocked with ice in the step of knocking, changing water in the step of scaling, eviscerating and cutting-up and changing the new sponge in the step of wiping fish with sponge.

CONCLUSION

Recently, the demand of Tabtim fish consumption has increased continuously because Tabtim fish is low price but high nutrition food. As a result, it affects the trend of both domestic and export consumption. One important problem that the importers concern for importing Tabtim fish was the microbiological quality of Tabtim fish. Therefore, in this research, the amount of bacteria contaminated in the chilled fresh Tabtim fish process, focusing on the bacteria causing alimentary canal illnesses such as Total Aerobic Bacteria, *Escherichia coli*, Coliform and *Staphylococcus aureus* were studied in order to assess the critical point processing. The results of the total aerobic bacteria and *E.coli* indicated that the highest contamination steps were the process that Tabtim fish was packed in polyethylene bags after chilled storage at 4°C for 7 days and that Tabtim fish was knocked which showed the level of contamination as 4.4×10^4 , and <10 CFU/cm² respectively. Therefore, total aerobic bacteria and *E. coli* results are not over the standard value in TACFS 7001-2004. While the amount of coliform and *Staphylococcus aureus* indicated that the highest contamination step was the process which Tabtim fish was wiped with sponge before packing which showed the level of contamination 6.6×10^3 and 3.4×10^2 CFU/cm², respectively. The results of this research will affect the safety of consumers and will help prolong the preservation time of chilled fresh Tabtim fish.

LITERATURE CITED

- AOAC, 1994. Association of Official Analytical Chemist, Official Method 990.12 Aerobic Plate Count in Foods. **AOAC International** 73: 242.
- AOAC, 2002. Association of Official Analytical Chemist, Official Method 998.08 *Escherichia coli* Count in Poultry, Meat and Seafood. **AOAC International** 82: 73
- AOAC, 2003. Association of Official Analytical Chemist, Official Method 2003.11 Enumeration of *Staphylococcus aureus* in Selected Meat, Seafood and Poultry. **AOAC International** 86: 947
- Aquatic Animal Research Center Charoenpokphand. 1999. **Tabtim fish culture. Technical paper** (Leaflet) 6 p.
- Curiale, M.S., T. Sons, J. Sue McAllister, B. Halsey and T. L. Fox. 1990. Dry Rehydratable Film for Enumeration Total Aerobic Bacteria in Foods: Collaborative Study. **J. Assoc. Off. Anal. Chem.** 73(2): 242-247
- Gangar, V., M. S. Curiale, K. Lindberg and S. G. Lenarz. 1999. Dry rehydratable film method for enumerating confirmed *Escherichia coli* in Poultry, Meat, and Seafood: Collaborative Study. **AOAC International** 82(1): 73-78
- Horwitz, W. and G. W. Latimer. 2005. Official Methods of Analysis 18th Ed., **AOAC International**, Gaithersburg, Maryland. 11p.
- 3 M Thailand Co.Ltd. 2005. Food Microbiology Technical Analysis. Technical paper. **Division of Microbiology**. 151 p.
- Sangjindawong, M. 1995. **Microbiology of Fishery Products**. Sahamit-opset Co.Ltd. Nontaburi. 238 p.
- Thai Agricultural Commodity and Food Standard (TACFS). 2004. Tilapia. **National Bureau of Agricultural Commodity and Food Standards (ACFS)**. TACFS 7001-2004. 8P.
- Watanasinthu, S. 2000. Hand Book of Food safety (Pocket book). **Food Science and Technology**. 74 p.