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Investigation for Contamination of Parasite and Aerobic Bacteria in Frozen Tilapia Fillets in Taiwan

JYH-WEI SHIN* AND YIN-HUNG HUANG

Division of Parasitology, College of Medicine, National Cheng Kung University
No. 1, Dashiue Rd., Tainan, 701 Taiwan, R.O.C.

ABSTRACT

Three hundred frozen tilapia fillet samples obtained from shopping malls, supermarkets and conventional markets from different geographical areas of Taiwan in the period July 1997 to June 1998 were screened for parasite and microorganism infections. Results showed that no samples were infected with parasitic metacercaria or larva, nor with *Vibrio*. However, 0.36% test samples were found to be contaminated with *Micrococcus* or *Aeromonas*. The average aerobic bacteria counts for all the test samples were 1.2 x 10^5 CFU/g; samples obtained from northern, central and southern Taiwan were 7.8 x 10^4, 1.4 x 10^5 and 1.5 x 10^5 CFU/g, respectively, and samples collected from shopping malls, supermarkets and conventional markets were 1.1 x 10^4, 1.4 x 10^5 and 1.5 x 10^5 CFU/g, respectively.

Key words: **frozen tilapia fillet, parasite diagnosis, aerobic bacteria count.**

INTRODUCTION

People consuming omophagia or rare-cooked fish meat are vulnerable to parasite infection. *Clonorchis sinensis* is a parasite which commonly infects fish meat in Taiwan. The first report in Taiwan regarding a *Clonorchis sinensis* infection was presented by Choi[1]. Investigation of *Clonorchis sinensis* infection by Chow[2], Kim and Kuntz[3], and Cross[4] reveals infection rates ranging from 0% to 57%. Three major infection areas in Taiwan are the Meinung area in Kaohsiung County[2,5-7], the Miaoli area[6,8] and the Sun-Moon lake area in Nantou County[6,9]. *Clonorchis sinensis* infection rates in the above areas are 10 to 52%[2,10-12], 57%[8] and 39 to 51% depending on the time and location of the investigation[9], respectively. In addition to *Clonorchis sinensis* parasite, *Heterophyes heterophyes*, *Metagonimus yokogawai*, *Diphyllobothrium latum*, *Gnathostoma spinigerum* and *Anisakis* have also been reported as parasites contaminating the fish products of Taiwan.

Tilapia is one of the major fish raised in Taiwan. It is popular because it is easy to grow, the meat is tasty, the price is cheap, and it is easy to cook. Tilapia is sold as whole fish or frozen fillet, and often called “live sea bream” for sashimi. During feeding or meat processing, the fish or final product is susceptible to parasite larva infection. In addition, other hygienic issues such as fish spoilage during storage should be concerned.

Correspondence to: Jyh-Wei Shin
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Health risks resulting from hygienic problems could cause a hypersensitive effect on the human body, a detrimental effect on the digestion system, or possible death.

According to a report from the ROC Department of Health, 1,754 food poisoning cases occurred in Taiwan from 1981 to 1998. Among them, 156 (8.9%) cases were related to seafood or seafood products. Therefore, the inspection of food microorganisms is extremely important. Wang *et al*.[14] have found that microorganisms and some chemical compositions in fresh tilapia and milkfish are not significantly different after the fish has been stored at 4˚C for 4 days or frozen at -15˚C. An investigation of microorganisms in tilapia and milkfish obtained from fish ponds, supermarkets and conventional markets was conducted by Chow *et al*.[15], and found that *E. coli* infection reached up to 100% due to water pollution in the fish ponds. Both food quality and safety have received great attention recently with the increased popularity of dining out. In this study, the investigation of parasites as well as microorganism infection in frozen tilapia fillets was conducted in order to prevent those fish products from contamination and protect the consumers from the risks of eating contaminated food.

MATERIALS AND METHODS

I. *Source of Samples*

All test samples were randomly collected from shopping malls, wholesale centers, supermarkets and conventional markets in northern (above Hsinchu), central (from Hsinchu to Chiayi) or southern (below Chiayi) Taiwan. The test samples were shipped in a cooler, which contained dry ice as a coolant, and placed in frozen storage in the laboratory. Inspection for parasites and microorganisms was done within 3 days of sampling.

II. *Parasite Inspection*

Two methods were used to inspect the samples for parasites. Test samples were digested and sampled as follows:

(I) *Sample Digestion*

The frozen tilapia fillet samples were defrosted at 4˚C overnight, sliced and then placed into a flask where a ratio of 1: 10 artificial gastric juice consisting of 1000 mL of saline solution, 7 g pepsin (Sigma, U.S.A.) and 10 mL of hydrogen chloride was added. The digestion procedure was performed at 37˚C for 6 hr. The digestion solution was filtered for removing undigested portions. The filtrate was then left to stand for 30 min. One third of suspension was decanted and refilled with saline solution. The mixture was then left to stand for a short time. This process was repeated until the suspension was clear. The clear suspension was then poured into a precipitation tube and allowed to stand for 1 hr. After removing the suspension, the precipitate was transferred into a Petri dish where a trace amount of saline solution was then added. The Petri dish was then swirled by hand and the parasite larva was observed in a central Petri dish.

(II) *Preparation of the Paraffin Slide*

A small portion of the test sample was fixed with formalin solution for 12 hr. An un-periodic shaking was performed during fixation of sample. The test sample was then dehydrated with alcohol, clarified with xylene, and immersed into a melted paraffin. The paraffin inclusion was then sliced into 5 μm thick samples and stained with a hematoxylin-eosin solution.

III. *Aerobic Bacteria Counting*

A mixture of 11 g test sample and 89 mL of saline solution was homogenized and diluted to a series of concentrations. Each dilution was cultured in a plate count agar (ADSA, Spain) at 37˚C for 24~48 hr.

IV. *Inspection of Vibrio*

The above homogenate was spread-cultured on a thiosulfate-citrate-bile salts-sucrose agar (TCBS agar, ADSA, Spain). A colony of the candidate was enriched at 30˚C for 24 hr, Gram-

Table 1. Inspection of aerobic bacteria count of frozen tilapia fillets from different sources in Taiwan

Sampling source	No. of Samples	No. of sample (%) in the range of aerobic bacteria count (CFU/g)			
		$<1x10^4$	$1x10^4$~$1x10^5$	$1.1x10^5$~$3x10^6$	$>3x10^6$
Mall	197	53(27.0)	110(55.8)	34(17.2)	0(0.0)
Supermarket	48	21(43.8)	19(39.6)	7(14.6)	1(0.4)
Conventional market	30	16(53.3)	9(30.0)	5(16.7)	0(0.0)
Total	275	90(32.7)	138(50.2)	46(16.7)	1(0.4)

stained and then identified with a Biolog system[13].

RESULTS

The purpose of this study was to investigate the contamination of parasites and microorganisms in commercial frozen tilapia collected from shopping malls, supermarkets and conventional markets in northern, central and southern Taiwan from July 1997~June 1998.

Results showed no parasite infection in any test samples. However, the average aerobic bacteria count for all test samples was 1.2 x 10^5 CFU/g; for samples obtained from northern, central and southern Taiwan 7.8 x 10^4, 1.4 x 10^5 and 1.5 x 10^5 CFU/g, respectively; and for samples collected from shopping malls, supermarkets and conventional markets 1.1 x 10^4, 1.4 x 10^5 and 1.5 x 10^5 CFU/g, respectively. These results also showed that the aerobic bacteria counts in 228 test samples (83%) were less than 1.0 x 10^5 CFU/g. However, 46 samples (16.7%) were found to have aerobic bacteria counts in the range of 1~3 x 10^6 CFU/g, and only 1 out of 275 test samples (0.4%) was detected to be over 3 x 10^6 CFU/g. No sample was found to be contaminated with *Vibrio*, and only 0.36% test samples were infected with *Micrococcus* or *Aeromonas*.

DISCUSSION

The commercial “live sea bream” fillet is made from sea-cultured tilapia after being treated through the following process: removing the head, organs and skin; individually quick freezing the fillet; vacuum packing or filling package with nitrogen gas to retard food spoilage, and then shipping to markets. This product is available at shopping malls, wholesale centers, supermarkets and conventional markets. Products with deterioration, discoloration, malodor or parasites do not meet the sanitation standards for frozen food[16]. Because food is susceptible to microorganism or parasite contamination during food processing, the sampling for this investigation of parasite and microorganism contamination in tilapia included the shopping malls, wholesale centers, supermarkets and conventional markets in northern, central and southern Taiwan. This investigation could provide valuable information concerning food safety for consumers.

There was no parasite infection found in the test samples. This could be due to the removal of the head, scales and organs where parasites are likely to infect thus reducing the infection possibility. Although trace skin residue was found in some of the test samples, no parasite infection was found after paraffin slide inspection. However, quality control of the final product is very important.

The aerobic bacteria count could reflect the quality of food sanitation during manufacturing, shipping and storage, and also provides an index of food freshness. According to the sanitation standards for frozen food[16], the aerobic bacteria counts for frozen seafood and seafood for omophagia should be less than 3 x 10^6 and 1 x 10^5 CFU/g, respectively. The aerobic bacteria counts in frozen seafood regulated by France and Japan are 5 x 10^4 and 1 x 10^5 CFU/g, respectively. Our investigation showed 1 out of 275 test samples

(0.4%) had an aerobic bacteria count higher than 3 x 10^6 CFU/g; while 47 samples (17.1%) and 78 samples (28.4%) had an aerobic bacteria count higher than 1 x 10^5 and 5 x 10^4 CFU/g, respectively. Because the food sanitation standards of some foreign counties are stricter than those of the R.O.C., the quality control should be stringently monitored for exporting frozen seafood. The aerobic bacteria count for fish obtained from conventional markets was the highest, followed by fish purchased from supermarkets and shopping malls. This could result from the method of storage. In conventional markets, the fish is put on ice and exposed to air. There is no labeling for the date of manufacturing and expiration, and the freshness of the fish is not guaranteed. Fish in supermarkets could possibly be repacked and the manufacturing date on labels could be changed to the date of repacking. Thus, the shelf-life of frozen fish could vary from several days to several months. Frozen "live sea bream" fillets in shopping malls or wholesale centers are directly obtained from seafood manufacturers, and are clearly labeled with the manufacturing and expiration date. Their shelf-life at -18˚C could be up to 1 year. Microorganism residue in frozen food could also grow during defrosting. The temperature rapidly reaches 0˚C and stays at that temperature for a period of time during defrosting. This process provides an opportunity for chemical reaction, recrystallization and microorganism growth. In conventional markets where the fish is kept on the ice, the temperature is not low enough to prevent microorganisms from growing. That is why the aerobic bacteria count in fish obtained from the conventional markets is the highest.

Food with a lower aerobic bacteria is not necessarily safer to consume as the food pathogen may still exist in the food with lower aerobic bacteria count. Lowering the temperature could inhibit the pathogen as well as other microorganisms. In some cases, using the aerobic bacteria count as an index for food sanitation is not adequate. Coliform is a microorganism that can cause food infection inducing sickness in the digestion system, and is unavoidable without proper sterilization. However, food with a high coliform count is not acceptable on the basis of food sanitation standards.

Vibrio and *Staphylococcus* were the two pathogens which most frequently caused the food poisoning in Taiwan from 1986 to 1995. Food poisoning outbreaks caused by the above two pathogens were weighed by 35.5% and 30.5%, respectively[17]. In this study, no *Vibrio* was found in test samples when using the TCBS spread-culture.

Clean food, rapid processing, heating and cold storage are the principles for quality control of processed food to ensure food safety. To achieve this goal, the Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Point (HACCP) standards are obeyed by most food manufacturers around the world. In Taiwan, affordable feeding costs, stable prices and fish supplies, and the freshness of raw material will make frozen tilapia one of the most profitable frozen seafood in the future.

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市售冷凍吳郭魚片寄生蟲與微生物調查

辛致煒* 黃尹虹

國立成功大學醫學院醫學系寄生蟲學科
台南市東區大學路1號

摘 要

本研究主要是針對市售冷凍吳郭魚片進行寄生蟲寄生感染情形及微生物分布調查。自民國86年7月起至87年6月止共計抽樣調查300件冷凍吳郭魚片樣品，分別購自北（新竹以北）、中（新竹至嘉義）、南（嘉義以南）三區之大型購物中心或量販店、生鮮超市及傳統市場，檢驗其寄生蟲寄生感染情形及進行衛生菌之檢測。寄生蟲寄生感染之檢驗結果皆爲陰性。衛生菌調查則包括好氣性總生菌數與弧菌檢測，結果顯示平均好氣性總生菌數爲1.2×10^5 CFU/g，依地區劃分，北、中、南三區平均總生菌數分別爲7.8×10^4、1.4×10^5及1.5×10^5 CFU/g；依購買地點劃分，大型購物中心、生鮮超市及傳統市場之平均總生菌數則分別爲1.1×10^5、1.4×10^5及1.5×10^5 CFU/g。腸炎弧菌之檢測結果皆爲陰性。此外，有0.36%的樣品被發現有微球菌屬（*Micrococcus*）或產氣單胞菌屬（*Aeromonas*）存在。

關鍵詞：冷凍吳郭魚片，寄生蟲檢查，好氣性總生菌數。