DEPARTMENT OF FISHERIES (MINISTRY OF AGRICULTURE)

FISH MARKETING ORGANIZATION (MINISTRY OF AGRICULTURE)

OFFICE OF THE ATOMIC ENERGY FOR PEACE

APPLIED SCIENTIFIC RESEARCH CORPORATION OF THAILAND

COOPERATIVE RESEARCH PROGRAMME NO. 23

PRESERVATION OF FISH AND FISHERIES PRODUCTS

RESEARCH PROJECT NO. 23/1

PRESERVATION OF FISH BY BOILING

REPORT NO. 2
BACTERIOLOGICAL STUDIES OF THE BOILED FISH INDUSTRY

BY

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F O R E W O R D

The title of this cooperative research programme has been broadened to cover 'Preservation of fish and fisheries products" from the earlier more restricted title of "Use of antibiotics for food preservation". At the same time the title of Research Project No. 23/1 has been changed to "Preservation of fish by boiling" instead of "Use of antibiotics for the preservation of fish, shell fish, and fishery products".

The present report is the second in the series started with Report No. 1 on Research Project No. 23/1 "Boiled Fish. I. Studies of the traditional method of preservation".

BOILED FISH. II. BACTERIOLOGICAL STUDIES OF THE BOILED FISH INDUSTRY

By Malee Sundhagul,* Pijitr Sornsrivichai,+ and Puangpen Smanmathurapoj*

SUMMARY

Boiling of fish in brine is a method of short term preservation of fish practiced in south-east Asia regions including Thailand. The keeping quality of boiled fish depends on, among other things, the bacteriological quality of the finished product. Low-quality raw materials with high bacterial counts together with unsanitary processing practices influence the quality of the boiled fish.

In this study the bacteriological quality of the raw materials such as fresh fish, salt, and water used in the preparation of the brine were examined. Sanitary conditions in the processing and marketing of boiled fish were investigated based on observation and bacteriological studies. Results of investigations are presented. Based on information obtained, processing steps and practices that tend to cause large increases in the bacterial content of the boiled fish are revealed. Suggestions to improve the sanitary conditions of the boiled fish industry are also given.

INTRODUCTION

Boiled fish is fish preserved by heat treatment under normal air pressure. The fish is cooked, after being gutted, in saturated brine. Boiling of fish is considered a simple and practical method of preserving fish for short periods under tropical conditions. It is practiced on a commercial scale in many countries of south-east Asia including Thailand (Rao 1965), where facilities for handling, transportation, and distribution are somewhat limited.

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Principally, the mackerel (*Rastrelliger* sp.) is used in making boiled fish, but other species, preferably of smaller size, such as Pla Nam-dok-mai (*Sphyraena* sp.) and Pla Hang-khaeng (*Caranx* sp.) may also be used. In 1965, more than 10 per cent of the mackerel caught were sold as boiled fish (Department of Fisheries 1966).

Since boiled fish has a high percentage of moisture (62 per cent) and a low percentage of salt (3-5 per cent), it can be kept for only two days under room temperature. The shelflife can be increased to one week if the treatment is repeated. The twice-boiled fish, however, is inferior in quality due to higher salt content (7-9 per cent) and tougher texture.* The spoilage of boiled fish is caused, mainly, by bacteria. The degree of spoilage is influenced by the number of spoilage bacteria. Their proteinase activity becomes significant when the number of spoilage bacteria approaches 10^7 bacteria per gramme of fish (Liston 1965).

Because of the universal use of bacterial counts as an index of sanitation, it is believed that bacteriological studies of the industry will provide useful information to evaluate processing practices and plant sanitation. The examination of production-line samples will, it is hoped, pinpoint processing steps that tend to cause large increases of undesirable micro-organisms.

MATERIALS AND METHODS

Total aerobic bacterial counts were made using tryptone-glucose-extract agar after 48 hours of incubation at room temperature ($27 \pm 3^{\circ}C$). Lactose broth and violet-red-bile agar were used to test for coliform bacteria and the results were read after 24 hours of incubation at $37^{\circ}C$. All media used were prepared from Difco dehydrated media (Difco Manual 1958). Pour plate method was used in all counts. The dilution samples were prepared by homogenizing the whole fish in sterile distilled water (1:3 w/v) in a waring blender. Four ml of fish homogenate was then added to 96 ml of sterile distilled water to obtain 10^{-2} dilution.

Fish samples were collected in plastic bags and stored under ice during

* "Boiled fish. I. Studies of the traditional method of preservation" by Malee Sundhagul. Report No. 1 on Research Project No. 23/1 (Preservation of fish by boiling). ASRCT unpublished report.

transportation to the laboratory. Water, salt, and brine samples were collected in sterile jars and stored under ice.

RESULTS AND DISCUSSION

Microbiology of raw materials

Usually, fish is transported by boat or truck to the processing plants. For short distance transport of less than 5 or 6 hours ice is not used and the fish is loaded directly onto the truck floor. For long distance, however, fish is iced and transported in wooden boxes. When freshly caught fish is not available, frozen fish is used for making boiled fish. At the plant the fish is unloaded, dumped onto the floor of the plant, exposed there in piles, and kept without ice. Because of poor handling, high counts are often found in raw materials of good quality. Table 1 shows the bacterial counts of raw fish upon delivery.

TABLE 1

TOTAL BACTERIAL COUNTS OF FRESH FISH

Conditions of delivery	Bacterial no. per g fish		
	Average*	High	Low
By boat, in the fish holds	1.3×10^5	3.3×10^5	2.4×10^4
By trucks, uniced	1.3×10^6	1.9×10^6	9.8×10^5
By trucks, iced and in the boxes	3.7×10^5	4.4×10^5	2.8×10^5
Thawed frozen fish	1.1×10^6	2.3×10^6	9.9×10^4

It can be seen that the total number of bacteria per unit weight of fish varies considerably. The bacteriological quality of the raw materials used in the production of boiled fish, thus varies. This in turn, will influence the quality of the finished product.

Icing of fish immediately after catching and during transportation, as in the case in delivery by boat, prevented the rapid increase of bacteria. Frozen fish after thawing showed unusually high average counts and much wider range of bacterial loads. This might be due to the fact that thawing was done at a slow rate and that often fresh fish before being frozen varied widely in

* Average number from five lots of fish.

bacteriological quality. Furthermore, it was not uncommon to use river water to speed up the thawing process and to thaw on a floor which was not cleaned.

The salt used for salting fish before boiling and for preparation of the brine for cooking the fish is unrefined solar salt of granular type. Solar salt produced in Thailand is extracted from sea water in eastern and southern shore regions. It is not pure and often shows discolouration. Impurities such as dirt particles are also common. Salt is usually stored in large wooden or cement tanks. Bacterial analysis of salt obtained from three different plants in Bangkok showed only slight variation in number of total bacteria (Table 2). The number of bacteria shown represented only salt-tolerant bacteria able to grow best in a medium having little or no salt, such as the tryptone-glucose-extract agar used in this study. Halophilic bacteria were deliberately ignored since they would not be able to grow on the finished product because of its low salt content.

TABLE 2

VARIATION IN NUMBER OF BACTERIA OF SALT

Source	Total no. bacteria per gramme
Plant A	3.2×10^4
Plant B	2.2×10^4
Plant C	4.3×10^4

The relatively low count of bacteria of salt revealed the common practice of selection of good-quality salt by boiled-fish producers for use in making boiled fish. The criteria normally used in grading the quality of salt are whiteness and absence of visible dirt particles.

Microbial changes during processing

Production-line samples of two plants were studied for changes in bacterial numbers. From each plant, all samples were collected from the one lot of fish because of the possible variation in the bacterial numbers of the raw materials. The collection was made after about two hours of daily operation. The flow sheet of the production-line is shown in Figure 1.

Fish, fresh or thawed

↓

Gills & entrails removed

↓

Washing

↓

Salting

↓

Immersion in saturated brine

(30 min)

↓

Cooking

Boiling in saturated brine

(10 min)

↓

Packing after cooling

Figure 1. Flow sheet of the boiled fish production-line.

Upon delivery, fish is unloaded onto the floor of the plant and the ice, if any, removed. Dressing of the fish by removing the gills and intestines through the mouth is also done on the floor in the same area. This practice results in recontamination of the fish by the drippings. Thus, removal of intestines did not reduce the number of bacteria as had been expected. Disposal of the guts and the gills directly into wooden tubs or plastic containers away from the fish might be introduced as a better sanitary practice at this step. Table 3 shows the changes of total bacterial counts at different steps during processing.

Washing of the fish after dressing is done in a wooden tub of water. After several washings the water is discarded and replaced with fresh water. Running water is sometimes used to dilute the wash water while the washing is being done, as in Plant I. For plants situated near the river or the shore, as Plant II, fish is washed in river water placed in the wooden tubs. In either case, however, the bacterial load was reduced to a certain extent. Use of clean water and frequent changes of water would undoubtedly, reduce further the number of bacteria of the raw materials to be introduced into the cooking

TABLE 3

CHANGES OF TOTAL BACTERIAL COUNTS DURING PROCESSING

Step during processing	No. of bacteria per g of fish	
	Plant I	Plant II
Fresh fish before processing	3.3×10^5	7.3×10^6
Fresh fish after removal of gills and intestines	6.9×10^5 (+)	1.4×10^7(+)
Dressed fish after washing	4.1×10^5 (-)	2.0×10^6 (-)
Dressed fish after mixing with salt	1.3×10^5 (-)	1.4×10^6 (-)
Salted fish before cooking	3.6×10^5 (+)	1.6×10^6 (+)
Boiled fish after boiling	2.3×10^3 (-)	8.2×10^5 (-)

brine and, finally, on the finished products.

Salting by mixing with salt and immersing in brine also reduced the bacterial load of the fish. Improper handling of the fish after the salting period, however, resulted in increasing number of bacteria. It was observed that the fish were placed on the plant floor after being taken out of the salting vat and before arranging them on bamboo trays or baskets for boiling.

Although boiling time may be as long as 10 minutes or sometimes longer, the cooking brine stays at the boiling temperature only for a very short time. As soon as the brine begin to boil more fresh brine is added. This practice of regulary adding more brine into the cooking pot helps to recontaminate the products and increase the load of bacteria of the cooking brine. Furthermore, the use of river water in the preparation of the brine for cooking and salting as practiced at Plant II resulted in much higher number of bacteria of the finished products. Here again, the use of clean water and pre-boiling of the cooking brine together with more frequent changes could result in better quality products with lower bacterial load. At Plant I, the cooking brine is changed daily at the end of the operation time. At Plant II, however, the changes are

(+) and (-) designate the increase or decrease of bacterial number from the previous step respectively.

made occasionally after several days of operation. Table 4 shows the variation in the bacterial numbers of the brines and washing water used in Plant I and Plant II.

TABLE 4

THE BACTERIAL COUNTS OF COOKING BRINE AND WASHING WATER

	No. of bacteria per ml	
	Plant I	Plant II
Freshly prepared brine	2.0×10^4	1.3×10^5
Boiling brine during operation	1.6×10^3	6.8×10^3
Washing water during operation	1.0×10^5	7.4×10^5

From Tables 3 and 4, it can be stated that high initial counts of raw materials and improper handling during processing contributed significantly to the high bacterial counts of the products.

Transportation time to inland markets varies from a few hours to one or two days. During transportation and storage, the number of bacteria increased very rapidly and reached significant levels of the order of 10^6 per gramme of fish after two days. Table 5 shows the changes of bacterial numbers of boiled fish during storage at room temperature (30 ± 3^oC).

TABLE 5

CHANGES OF BACTERIAL NUMBERS OF BOILED FISH
DURING STORAGE AT ROOM TEMPERATURE

Time of	Bacterial number/g of fish		
storage day(s)	Lot I	Lot II	Lot III
0	7.0×10^3	4.2×10^2	1.5×10^4
1	1.6×10^5	-	1.1×10^5
2	6.0×10^5	9.9×10^4	1.4×10^5
3	2.5×10^6	6.6×10^5	2.3×10^7

In addition of the total number of bacteria, the presence of coliform organisms also indicates the unsanitary nature of the products. Tests for the presence of these organisms on boiled fish sampled at random from the local markets in the Bangkok metropolitan area showed that the products for sale were, in general, free of these organisms. The positive result indicating their presence was found only in one case. Results were shown in Table 6.

TABLE 6

RESULTS OF COLIFORM TEST OF BOILED FISH

Sample no.	Source	Presence of coliform organisms	Bacterial no. per g of fish
1	Boiled fish plant	-	1.0×10^3
2	same	-	1.3×10^3
3	Bangrak market	-	6.0×10^2
4	Saphan Kwai market	-	1.9×10^4
5	Banglampoo market	+	2.5×10^4
6	same	-	1.2×10^4
7	same	-	2.6×10^4

Contamination by coliform micro-organisms during selling period was rare but still possible by direct contact of the products with the hands of the sellers or the buyers, usually when one or two fishes were purchased. Normally, boiled fish are handled by a tray of three or four fishes, thus avoiding direct contact of the products by hands. As shown in Table 6, freshly boiled fish had no coliform organisms. On the other hand, fresh fish before boiling almost always gave positive results to the coliform test. Results of the tests on fish before and after processing were shown in Table 7.

TABLE 7

THE PRESENCE OR ABSENCE OF COLIFORM ORGANISMS
OF FRESH AND BOILED FISH

Description of samples	Lot I	Lot II	Lot III
Fresh fish, whole, unwashed	present	present	present
Fresh fish, gutted, unwashed	present	present	present
Fresh fish, gutted, washed	present	absent	absent
Fresh fish, salted	absent	absent	absent
Freshly boiled fish	absent	absent	absent

CONCLUSIONS

1. The quality of raw materials used in the production of boiled fish influence that of the finished products. Partly spoiled fish with high bacterial numbers made products of low keeping quality. Low grade salt containing dirt and dust particles introduced into the dipping solution large numbers of bacteria. In addition, it could result in unattractive appearance of the products. Proper handling and icing of fresh fish during transportation would result in better-quality raw material.

2. Studies of the changes in bacterial numbers during processing revealed that washing fresh fish, after being gutted, helped to reduce the number of bacteria of the fish considerably. Improper handling of the fish during processing reintroduced bacteria onto the fish while proper disposal of gills and intestines during dressing prevented recontamination of the gutted fish. Use of clean running water or frequent changes of washing water could improve the quality of the product through reduction of bacterial number. Removal of the finished product from the processing area should also help to prevent recontamination of the finished product.

3. Coliform organisms which might be present in fresh fish were destroyed by salting and heat treatment. Recontamination of the finished products during

marketing by flies or direct contact with hands could be prevented if the products on display were covered to keep off flies and not picked up individually by hands.

4. In general, the traditional method of boiling fish in brine was found to be quite effective in reducing the number of bacteria of the fish and, thus, increased the shelflife of the fish for several hours at room temperature. If ways could be found further to reduce the bacterial number and to prevent recontamination, it would be possible to increase the shelflife even further. Results of the present studies reveal some of the causes of high bacterial counts and the sources of recontamination. The information obtained points to ways of improving the keeping quality of the products through improving the quality of raw materials, and avoiding practices that tend to cause large increase of bacteria on the fish.

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