

BACTERIOLOGICAL EVALUATION OF FROZEN RAW FISH PRODUCTS

By

JAN GJERDE*

Institute of Vitamin Research
Directorate of Fisheries, Bergen

ABSTRACT

Two hundred samples of frozen fish products were examined bacteriologically. The majority of the products analyzed were of acceptable bacteriological quality. The aerobic plate counts ranged from 1×10^4 per g to 4.5×10^6 per g. Faecal streptococci were found in 53 % of the samples, faecal coliforms were found in 16 % and *Escherichia coli* were found in 10 % of the analyzed samples. Coagulase positive *Staphylococcus aureus* and sulphite reducing clostridia were detected in few samples and in low numbers per g.

INTRODUCTION

It is established that sanitary conditions during processing of food products are reflected in the bacteriological content of the finished products (PHILLIPS and PEELER 1972, ZAPATKA and BARTOLOMEO 1973). Useful bacteriological data for raw frozen fish products have been presented (JØRGENSEN 1962, NICKERSON et al. 1962, NEUFELD 1968, SURKEIEWICZ 1968). Little information, however, is available about frozen fish products on the Norwegian market. This survey was made to investigate the bacteriological quality of frozen fish products produced by Norwegian companies.

The Norwegian frozen fish products are supplied with fish as raw material from smaller boats unloading their catch frequently, and larger vessels which can keep their catch up to ten days before unloading. The fish are gutted, eviscerated and refrigerated with ice on board. In the factory the fish are filleted and prepared in consumer size packages and frozen. The fish factories operate with different hygienic facilities, according to building construction, equipment for refrigeration and clean water supply.

* Present address: Central laboratory, Directorate of Fisheries

MATERIALS AND METHODS

SAMPLING :

A total of 200 samples of commercially frozen fish products were collected during one year. The samples consisted of consumer packed fish fillets and breaded fish from the main producers on the Norwegian market and represented an average of frozen fish products. The products were brought to the laboratory immediately after sampling and stored in a deep freezer until the bacteriological examination was carried out. One hundred grams of the frozen samples were aseptically removed and minced. From the minced meat three parallel samples of 10 grams each were homogenized in 90 ml sterile physiological saline in a Serwall Omnimixer and serial decadic dilutions were made in the same diluent.

Analytical procedure : The AOAC (Association of Official Analytical Chemists 1970) procedure was used with slight modifications to determine the aerobic plate count (APC), the most probable number (MPN) of faecal coliform and *Escherichia coli*.

Methods of the Nordic Committee on Food Analysis ,no. 68 and no. 56, were used to determine the number of faecal streptococci and sulphite reducing clostridia.

Coagulase positive staphylococci were detected on Baird Parkers medium according to The Oxoid Manual 1965.

DESCRIPTION AND MODIFICATIONS OF THE TESTS :

1. Aerobic plate count was determined on Plate count agar, (Oxoid Cm 183). Duplicate plates were incubated at 20°C for 4 days and at 37°C for 2 days.
2. Faecal coliforms were determined by using five tubes for each dilution incubated at 37°C in Lauryl sulphate broth (Merck no. 10266). Eijkmans lactose broth (Difco 0017-01) was used for the confirmative procedure and incubated at 44.5°C from which the faecal coliforms were estimated.
3. *Escherichia coli* was determined by streaking from Eijkmans lactose broth tube showing gas to Levine eosine methylene blue (EMB) agar (Oxoid Cm 69) and incubating at 37°C. Further identification was done according to the AOAC procedure.
4. Faecal streptococci counts were estimated on Enterococcus agar (Difco no. 0746-01) by surface smear inoculation. Typical colonies were counted after incubation for 72 hr at 37°C (NMK no. 68, 1968).
5. Sulphite reducing clostridia counts were estimated on Iron sulphite agar in high agar tubes. Characteristic colonies were counted after 4 days incubation at 37°C (NMK no. 56, 1965).

6. Coagulase positive *Staphylococcus aureus* was estimated by surface smear inoculation on Baird Parkers medium (Oxoid Cm 275). The plates were incubated for 48 hr at 37°C, and characteristic colonies were tested for coagulase activity using rabbit plasma (Oxoid manual, 1965).

RESULTS AND DISCUSSION

The bacteriological tests performed were selected to determine levels of indicator bacteria and total counts as a monitor of the overall sanitation in the production of raw frozen fish products. It is possible to reduce the number of tests performed in ordinary food control. The samples examined in microbiological investigation of food usually represent a small part of the total production, and these results give only an indication of the number of microorganisms in frozen fish products available on the Norwegian market.

Table 1. Aerobic plate count in raw frozen fish products grouped in six count ranges.

Count ranges	APC per g at 37°C	APC per g at 20°C
0–1 × 10 ⁵	25 %	23 %
1 × 10 ⁵ –2.5 × 10 ⁵	31 %	28 %
2.5 × 10 ⁵ –5 × 10 ⁵	21 %	18 %
5 × 10 ⁵ –1 × 10 ⁶	18 %	17 %
> 1 × 10 ⁶	5 %	14 %
Geometric mean for all samples investigated	1.7 × 10 ⁵	2.6 × 10 ⁵
Min/max APC per g	3 × 10 ³ –1.8 × 10 ⁶	1 × 10 ⁴ –4.5 × 10 ⁶

Table 1 shows the results for APC at 20°C and 37°C. APC at 20°C ranged from 1 × 10⁴ per g to 4.5 × 10⁶ per g with a geometric mean of 2.6 × 10⁵ per g. APC at 37°C showed a similar pattern, but at a lower level. The APC at 37°C was found to be relatively high compared with parallel counts at 20°C. Because of the preponderance of psychrophilic bacteria in fish stowed in ice (SPENCER 1961), the results indicate that the fish have been exposed to higher temperatures.

The APC at 20°C showed considerable variation. Compared with the existing data on the content of microorganisms in frozen fish products at 25°C in international trade, some of the results in this investigation were found to be high. (The international Commission on Microbiological Specification for Foods, 1974).

In international trade more than 48% of fish fillet and frozen breaded fish portions had an APC lower than 1 × 10⁵ per g. Products with an

APC more than 1×10^6 per g were found in only 7 % of the products investigated (International Commission on Microbiological Specification for Foods). The APC of raw packed fish products will depend on the number of bacteria in the raw fish in addition to the bacterial contamination during processing.

The producers of frozen fish in Norway are supplied with fish which have been stowed in ice for one to ten days. Since the fish flesh will be invaded by bacteria during storage, it can be difficult for the companies to produce fish products with a low APC even if the processing is done under satisfactory hygienic conditions.

Table 2. Faecal coliforms, *E. coli*, faecal streptococci, sulphite reducing clostridia and coagulase positive staphylococci in raw frozen fish products grouped in five count ranges.

Count range	Faecal coliforms (MPN/g)	<i>E. coli</i> (MPN/g)	Faecal streptococci	Sulphite reducing clostridia	Coagulase positive staphylococci
0	84 %	90 %	47 %	93 %	97 %
0-50	12 %	10 %	8 %	7 %	3 %
50-100	4 %		25 %		
100-1000			9 %		
> 1000			11 %		
Max. count			7.2×10^3		

Table 2 shows that faecal coliforms were detected in 16 % of the products. In 12 % was found less than 50 per g and in 4 % between 50 and 100 faecal coliforms per g. Existing data for similar products in international trade show almost the same results. (International Commission on Microbiological Specification for Foods).

E. coli was found in 10%, and faecal streptococci were found in 53% of the products investigated. Faecal streptococci are normally more resistant against freezing injury and disinfectants than *E. coli* (RAJ et al. 1961, VARGA and ANDERSON 1968). In this investigation faecal streptococci were found in higher numbers and more frequently than *E. coli*. The origin of these bacteria, which are of sanitary significance, may be contamination either from fish handling rooms on the vessels or during processing of the fish in the factories. It has been shown that indicator bacteria, such as *E. coli* and faecal streptococci can multiply on working surfaces (VARGA and ANDERSON 1968) and detection of these bacteria are an index of general sanitation rather than direct faecal contamination.

Coagulase positive *Staphylococcus aureus* were only detected in 3% of

the samples and in low numbers per g. Sulphite reducing clostridia were also detected in few samples (7%) and in low numbers per g.

The majority of the samples of frozen fish products analyzed in this study were of acceptable bacteriological quality. In none of the samples were the upper limits of the proposed acceptable levels found to be exceeded. For the APC the limit proposed is 1×10^7 organisms per g, faecal coliform bacteria 4×10^2 per g and *Staphylococcus aureus* 2×10^3 per g.

However, recommendations for bacteriological food control of fish and fishery products propose that three out of five samples drawn from a group or lot should not exceed 1×10^6 per g for APC and 4 per g for faecal coliform bacteria. Following this proposition, some of the analyzed groups of products could possibly be rejected, and this would make it necessary to look more closely into some companies which are working under poor sanitary conditions.

REFERENCES

- AOAC. Association of Official Analytical Chemists 1970.
- JØRGENSEN, B. V. 1962. Bakteriologiske undersøkelser av detaljpakkede, frosne torske- og rødspettefileter. Bacteriological investigation of detailpacked, frozen fillets from cod and plaice. *Konserves og Dypfrost* 20, 94—99.
- NEUFELD, N. 1968. Influence of bacteriological standard on the quality of inspected fisheries products. *Fish Inspection and Quality Control*, 234—240, published by Fishing News (Books) Limited, Ludgate House 110 Fleet Street, London.
- NICKERSON, J. T. R., SILVERMAN, G. J., SOLBERG, M., DUNCAN, D. W. and JOSELOW, M. M. 1962. Microbial analysis of commercial frozen fish sticks. *J. Milk Food Technol.* 25, 45—47.
- Nordic Committee on Food Analysis (NMK), no. 69, 1968, no. 56, 1965.
- Oxoid Manual, Oxoid Limited, London 1965.
- PHILLIPS, F. A. and PEELER, J. T. 1972. Bacteriological survey of the blue crab industry, *Appl. Microbiol.*, 24, 958—966.
- RAJ, H., WIEBE, W. J. and LISTON, J. 1961. Detection and enumeration of faecal indicator organisms in frozen sea foods, II, Enterococci. *Appl. Microbiol.* 9, 295—303.
- SPENCER, R. 1961. The bacteriology of distant water cod landed at Hull. *J. Appl. Bacteriol.* 24, 4—11.
- SURKEWICZ, B. F., GROOMES, R. J. and SHELTON, jr. L. R. 1968. Bacteriological survey of the frozen prepared foods industry. IV. Frozen breaded fish. *Appl. Microbiol.* 16, 147—150.
- The International Commission of Microbiological Specification for Foods (ICMSF) of the International Association of Microbiological Societies, 1974, *Microorganisms in Foods 2. Sampling for microbiological analysis: Principles and specific application.*
- VARGA, S. and ANDERSON, G. W. 1968. Significance of coliform and enterococci in fish products. *Appl. Microbiol.* 16, 193—196.
- ZAPATKA, F. A. and BARTOLOMEO, B. 1973. Microbiological evaluation of cold water shrimp. *Pandalus borealis*. *Appl. Microbiol.* 25, 858—861.