REPORT ON MICROBIOLOGY QUALITY OF FISH BALLS

31ST DECEMBER 2004

Fish ball is one of the commonest fish products produced worldwide. In Malaysia, fish balls are normally marketed and stored by retailers on ice. This way of handling the product has been realized to be a contributory factor to the relatively short shelf life of fish balls. Observation by the author shows that within fish balls tend to develop slime within three day of production during retailing. It is therefore important that the microbiological quality (to determine associated bacteria) of such product be known in order to determine the specific procedures or methods to extend the shelf life of the product.

SAMPLES ANALYSIS:

1. FRESH FISH BALLS – SAME DAY PRODUCTION
2. SPOILT FISH BALL SAMPLES – PRODUCED 24TH DECEMBER 2004
3. SURIMI PASTE RAW MATERIAL
4. SWAB FROM GRINDING MACHINE
5. SWAB FROM CONVEYOR – POST CHILLING 1
6. SWAB FROM CONVEYOR – POST CHILLING 2
7. PRE-PACKING CONVEYOR

Samples and swabs were evaluated for Total Viable Count and Pseudomonas counts. Sixteen colonies were randomly selected from the Plate Count Agar for spoilt samples and Identified using the Biolog Microlog Systems for Identification of microorganisms. Spoilt samples were also evaluated for spore formers. However, none was detected. Table 1 shows the results of the various analyses for the respective samples.

Table 1: Total viable, *Pseudomonas* counts, coliform and Test for E. coli.

Samples	Total Viable Count cfu/g	Pseudomonas count cfu/g	Coliform (MPN/g)	Test for E. coli
Fresh fish balls (initial counts)	4.41×10^5	4.11×10^4	4.6×10^3	+
Spoilt fish balls	9.55×10^7	Not tested	Not tested	Not tested
Surimi paste	7.07×10^4	6.00×10^3	2.3×10^2	-
Grinding machine	1.38×10^4	2.35×10^3	30	-
Conveyor 1	6.92×10^6	7.8×10^3	91	-
Conveyor 2	3.47×10^6	6.35×10^4	2.4×10^3	-
Pre-packing conveyor	2.47×10^4	0	30	-

Bacterial types that were identified to be involved in spoilage and slime of the fish ball samples are shown in table 2:

Table 2: Bacteria types Identified in spoilt fish ball samples

Sample ID	Bacteria type	No of isolates
Spoilt Fish balls	*Pseudomonas putida*	3
	Other Pseudomonas spp	4
	Staphylococcus sciuri	3
	Acinetobacter calcoaceticus.	1
	Vibrio furnissii	1
SLIME	*Enterobacter gergoviae*	1
	Staphylococcus sciuri	1
	Vibrio furnissii	1
	Streptocuccus spp	1

Conclusion

Spoilage offish balls is due to *Pseudomonas* spp. as they are predominant. The question is where do the *Pseudomonas* come from?

Storage Studies

Objective:

Objective of the study was;

1. To determine spoilage and slime causing bacteria in Fish balls stored at 4 ºC, 10 C and in ice.

Storage studies were also carried out for 7 days at 4 ºC, 10 C and in ice (in a Coleman cool box). Ice was replaced twice a day. Total viable counts, *Pseudomonas* counts, spore formers and *Shewanella putrefaciens* counts were conducted on days 1, 3, 5 and 7 of storage. Tests were conducted for the presence of *E. coli* during the storage period. pH of fish balls were also measured during the storage period.

Microbiological assessment

10g of sample was stomached in 90 ml of 0.1% peptone water for 45 sec using a Lab Blender. Serial dilutions were prepared from the homogenized sample and 100 µl was inoculated onto appropriate agar using the WASP 2 spiral plater.

Total Plate Count was carried out using Plate count agar incubated at 35 ºC for 24-36 hours.

Total Coliforms & E. coli

Total coliforms were enumerated using LST broth and the 3-tube MPN method, incubated at 37 ºC for 48 hrs. Tubes showing positive results were further analyzed for the presence of E. coli. Positive tubes were inoculated into Brilla Broth and incubated at 44 ºC for 24 hrs. Brilla broth culture was then streaked on Eosin Methylene Blue Agar and incubated at 37 ºC for 24 hrs to determine growth indicative of *E. coli*.

Shewanella putrefaciens

For H_2S producing bacteria enumeration, typical of *S. putrefaciens*, a 1.0 ml sample was inoculated into 10 ml of molten iron agar. After setting, a 10ml overlay of molten medium was added and incubated at 20^oC for 4 days; black colonies formed by the production of H_2S were enumerated.

Pseudomonas spp.

Pseudomonads spp. were enumerated on Pseudomonas agar supplemented with cetrimide fusidin cephaloridine (CFC) incubated at 30 ^oC for 48-72hrs.

Aerobic Sporeformers

Enumeration of aerobic sporeformers was carried out using Tryptone glucose extract agar (TGE). Fish homogenates in 0.1% peptone water was heated at 80 ^oC for 30 min and then cooled. 1.0 ml of the cooled fish homogenate was transferred into 15-20 ml of molten TGE agar, mixed and then poured into Petri plates and incubated at 35 ^oC for 48 hrs.

Isolation and identification of spoilage/slime causing organisms

Colonies were selected randomly from plate count agar during the storage period, purified and identified using the biolog system. The selected colonies were streaked on tryptic soy agar twice and incubated at appropriate temperature for 24 hours.

Before identification, isolates were streaked unto Biolog Universal Growth agar and incubated for 24 hrs. Gram staining, oxidase test and catalase test were carried out on each isolate. Bacterial suspensions were prepared using biolog's GN/GP Inoculating fluid which was used to inoculate GN2 (for gram negative bacteria) or GP2 (for gram positive bacteria) MicroPlates and incubated at appropriate temperature (depending on bacteria type) for 24 hours and then read using the Biolog Microstation System (Biolog Inc, 2001) with its equivalent software and database to identify individual bacterium.

Results

pH of Fish balls during storage

The average pH of fresh fish balls was measured to be 6.83. pH of fish balls stored at 4, 10 and in ice are shown in Table 4. A pH decrease was observed at all storage conditions. However, pH of fish balls stored in ice was relatively lower than those stored at 4 and 10 ^{o}C at the end of the 7 days of storage. The question is does pH have any effect on gel stability of the fish ball which could result in the slime formation as a result of exudates which is in turn due to synersis.

Table 4 Internal pH of fish balls stored in ice, and at 4 ^{o}C and 10 ^{o}C for 7 days

Storage Condition	Day	pH
4 ^{o}C	1	6.91
	3	6.93
	5	6.87
	7	6.67
10 ^{o}C	1	6.87
	3	6.83
	5	6.34
	7	5.79
ICE	1	6.82
	3	6.47
	5	5.12
	7	5.01

***Aerobic Spore formers and Shewanella putrefaciens* counts**

No spore formers were detected during the storage period. *Shewanella putrefaciens* was detected on few occasions but only few colonies (less the 3) were observed even at the lowest dilution of 10^{-1}.

Total Viable, Pseudomonas and coliform counts

Table 3 show the results for TVC, Pseudomonas counts, Coliform and E. coli confirmation. Initial total viable count was 4.41 x 10^5. TVC increased in all fish balls stored at the different temperatures. However the rate of increase was slow for samples stored at 4 ^oC and in ice compared to fish ball stored at 10 ^oC. By the fifth day of storage, TVC of fish balls stored at 10 ^oC has exceeded 10^7, whiles TVC for fish balls stored at 4 ^oC and in ice were around 10^6.

Table 3: TVC, Pseudomonas counts and E. coli confirmation of fish balls during storage in ice, at 4 ^oC and 10 ^oC for 7 days.

Storage Condition	Day	TVC (cfu/g)	Pseudomonas count (cfu/g)	Coliform counts (MPN/g)	Test for E. coli
4 ^oC	1	5.44 x 10^5	4.75 x 10^4	2.25 x 10^3	-
	3	1.26 x 10^6	3.25 x 10^4	3.0 x 10^3	-
	5	6.45 x 10^5	2.00 x 10^4	6.35 x 10^3	-
	7	1.78 x 10^6	7.30 x 10^5	3.3 x 10^3	-
10 ^oC	1	7.44 x 10^5	6.55 x 10^4	9.1 x 10^2	+
	3	5.98 x 10^6	1.20 x 10^6	3.0 x 10^3	-
	5	2.22 x 10^8	1.79 x 10^8	3.6 x 10^3	-
	7	7.75 x 10^8	4.15 x 10^8	9.3 x 10^4	-
ICE	1	3.44 x 10^5	6.15 x 10^4	2.3 x 10^3	-
	3	4.16 x 10^6	3.03 x 10^6	4.9 x 10^4	-
	5	2.61 x 10^6	1.03 x 10^6	8.4 x 10^4	-
	7	2.36 x 10^6	1.77 x 10^6	3.6 x 10^4	-

Pseudomonas counts remain below 10^6 and 10^7 in fish balls stored at 4 ^oC and in ice respectively at the end of the 7 days storage, whiles that of fish balls stored at 10 ^0C exceeded 10^8. E. coli was only detected once during storage in fish balls stored at 10 ^oC. Coliform counts remained between 10^3 and 10^4 for all fish balls stored at the various temperature conditions.

Table 5: Bacteria types Identified in fish ball samples during storage in ice and at 4 ^oC and 10 ^oC for 7 days

Storage Condition	Bacteria type	No of isolates
4 ^oC	*Staphylococcus sciuri*	4
	Pseudomonas fluorescens biotype G	3
	Pseudomonas putida biotype A	1
	Brevibacterium epidermidis	1
	Enterobacter asburiae	1
	Micrococcus caseolyticus	1
	Acinetobacter genospecies 10.	1
10 ^oC	*Pseudomonas putida biotype A*	5
	Staphylococcus sciuri	3
	Pseudomonas bathycetes	1
	Raoultella terrigena	1
ICE	*Staphylococcus sciuri*	4
	Pseudomonas fluorescens biotype G	1
	Enterobacter cloacae	3
	Pseudomonas bathycetes	1
	Enterobacterasburiae	1
	Raoultella terrigena	1
	Klebsiella pneumoniae ss pneumoniae	1
	Chryseobacterium gleum/indologenes	2

Conclusion

From the results, it is shown that the initial total viable count for fresh fish balls is relatively high for such a cooked product. Since the fish balls went through a cooking process at a temperature of 90 – 95 ^{o}C, it is expected that most vegetative bacteria cells will be destroyed. Therefore, any bacteria growth after that might be due to growth of spores or post-cook contamination. However, from the results no spore formers were detected. Therefore, the possible source of bacteria in the fresh fish balls might be through cross- or re-contamination. This re-contamination could have occurred as a result of the use of contaminated chilled water/ice, conveyor belt, and through the handling of fish balls by operatives.

From the results it is clear that storage of fish balls at 4 ^{o}C was superior to other storage condition. The reason for the possible slow growth of bacteria in fish balls stored in ice, which is comparable to those stored at 4 ^{o}C, might be due to cold shock as a result of lowering of the temperature to around 3 ^{o}C when ice was replaced with fresh one. Before replacement of ice the temperature rises to about 15 ^{o}C.

The main bacterial types identified included *Pseudomonas spp* and *Staphylococcus sciuri*. Even though *Enterobacter cloacae* also showed up in fish balls stored in ice, it was not detected in fish balls stored at 4 and 10 ^{o}C. Both *Pseudomonas spp* and *Staphylococcus spp.* are present in the environment and in a case of poor decontamination of the food processing environment these can end up in the finished products. *Staphylococcus spp* in particular are also found on the skin of humans and these can be transferred into processed food when hygiene is compromised.

Recommendations

1. It is recommended that cleaning and sanitation within the processing area be improved, not forgetting personal hygiene of operatives.
2. There should also be a physical demarcation between raw material area and finished product area. Backwards movement of both products and operatives should be controlled to prevent re-contamination of finished products with raw materials.
3. The application of decontamination processes, such as use of in-plant chlorination or organic acids, in the chilling water could be tested and applied if possible.
4. Overall there is the need for improvement and development of sanitation/hygiene programmes to ensure overall quality of finished products.
5. The use of preservatives is not recommended as the study indicates that spoilage is due to lack of hygiene and post-process contamination.

Further study

Complete microbiological inventory of the plant must be carried out.