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Microbiological quality of *keropok lekor* during processing

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Abstract: *Keropok lekor* is an important fish product in Malaysia. The customer demands for *keropok lekor* have been increasing. This study was conducted to analyze the microbiological quality of *keropok lekor* in every stage of its processing, namely mincing, mixing, kneading, boiling and cooling. When processing *keropok lekor*, the boiling of *keropok lekor* at 100°C for 10 min reduced the Total Plate Counts (4.38±0.47 $\log_{10}$ cfu/g), psychrotrophic counts (2.00 ± 0.00 $\log_{10}$ cfu/g), mesophilic sporeformer counts (1.26 ± 0.34 $\log_{10}$ cfu/g) and total coliform counts (1.71±0.51 log Most Probable Number/g) significantly ($p>0.05$). However, the microbial counts were found to increase significantly ($p<0.05$) after the cooling process, except for the yeast and mold counts and *Staphylococcus aureus* counts. The presumptive predominant microorganisms, isolated before the boiling stage, were members of the *Enterobacteriaceae* family and those belonging to *Pseudomonas, Vibrio*, *Staphylococcus*, *Bacillus* and *Micrococcus*. After the boiling stage, the presumptive predominant microorganisms were members of *Enterobacteriace* family and those belonging to *Micrococcus*, *Bacillus*, *Staphylococcus* and *Aerococcus*.

Keywords: *keropok lekor*, microbiological quality, processing

Introduction

Keropok lekor is a popular and highly relished fish product in Malaysia. It is widely sold in the local market and usually produced on a daily basis to fulfil the market demand, especially for school canteens, night markets and hawker stalls. At present, most of *keropok lekor* manufacturers carry out the processing manually, following established procedures with little mechanization.

Keropok lekor is known to have a short shelf life of only one day at the room temperature (Embong *et al*., 1990). Che Rohani and Mat Arup (1992) showed that the total viable count of bacteria in *keropok lekor* increased from less than 1 x 10^2 cfu/g to 1.5 x 10^8 cfu/g after 2 days at the room temperature. Signs of spoilage for this product include sliminess and formation of spots on the surface, which are resulted from the bacterial growth. Thus, a good understanding of the microbial profile of *keropok lekor* is vital. Microorganisms gain access into processed meat, from a variety of modes including ingredients, environment, equipment and handlers, during processing. This will definitely affect the microbiological status of the product. Processing conditions such as boiling of product can reduce microbial levels, although recontamination takes place during post-processing and handling of food (Sachindra *et al*., 2005).

Pseudomonas, *Flavobacterium*, *Corynebacterium*, *Lactobacillus*, *Bacillus* and *Micrococcus* are the major spoilage microorganisms in fish products such as fish sausages (Sasayama, 1973). Both psychrotrophic and mesophilic bacteria have been isolated during the processing of fish products. The presence of *Staphylococcus aureus* in food usually indicates cross contamination and mishandling of the fish products (Ingham, 1991), while total coliform counts are normally used in food processing as indicators of hygienic (Jay, 2000).

To the present, information on microbiological quality of *keropok lekor* is still very limited. Therefore, the objectives of this study were to determine the microbiological profile of *keropok lekor* at different stages of processing (mincing, mixing, kneading, boiling and cooling).

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Materials and methods

Samples

Five replicates of 500 g samples were collected immediately after each processing stage, namely after mincing of fish meat, after mixing of all ingredients, after kneading the dough, after boiling and after cooling at ambient temperature. These samples were obtained using sterile utensils and placed in sterile plastic bags which were properly labelled and dated. The samples were then brought to the laboratory in a pre-chilled container with crushed ice (4±1°C) and analysed within 24 hour.

Microbiological analysis

These samples were analysed using the standard procedures (APHA, 2001). A 25 g of keropok lekor was aseptically transferred to a sterile stomacher bag and pummelled for 1 min in a stomacher (Seaward Stomacher 400, BA-7021), with 225 ml of sterile 0.1% peptone water. Appropriate decimal dilutions of the samples were prepared using the same diluents and plated in duplicates on different growth media.

Total plate counts (TPC) and Psychrotrophic counts were determined using the Plate Count Agar (PCA)(OXOID), incubated at 37°C for 48 hours and 7°C for 10 days, respectively. Yeast and mold counts were determined using the Potato Dextrose Agar (PDA) (OXOID) which was incubated at 32°C for 7 days. Mesophilic sporeformer counts were determined using the Dextrose Tryptone Agar (OXOID) which was incubated at 37°C for 48 hours, after heating the inoculated agar at 80°C for 10 min to destroy vegetative cells.

Staphylococcus aureus was enumerated using the Baird-Parker Agar (BPA)(OXOID) which was incubated at 37°C for 48 hours; while total coliform and fecal coliform by MPN method using the Brilliant Green Bile Broth (OXOID) which was incubated at 37°C for 48 hours and at 44°C for 48 hours, respectively (ICMSF, 1978).

In this study, all the results were expressed as $\log_{10}$ number of colony forming unit/ gram ($\log_{10}$ cfu/g), except for the total coliform and fecal coliform which were reported as $\log_{10}$ number of most probable number/ gram ($\log_{10}$ MPN/g).

Presumptive genus identification of bacteria isolated at different stages of keropok lekor processing.

A total of 230 isolates, from the PCA plate, were randomly picked at the different stages of *keropok lekor* processing. These isolates were identified up to the genus level according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1993). All the isolates were purified by streaking them on the Nutrient Agar (OXOID) and maintained on the Nutrient Agar slants (OXOID). The following biochemical tests (Appendix B and B1) were conducted: Gram reaction according to the Hucker's method; the cell morphology by phase-contrast microscopy (Gerhardt *et al*., 1981); catalase formation by dropping a 3% H_2O_2 solution directly onto each colony; cytochrome oxidase by Kovacs reagent (ICMSF, 1983; Mossel and Moreno Garcia, 1985); glucose metabolism according to Hugh and Leifson's method (Harrigan, 1998), arginine dihydrolase by Thornley-medium (Thornley, 1960) and motility by motility test medium (ICMSF, 1983).

Physicochemical analysis

Internal temperature was determined with portable thermometer (Maxthermo) at the geometric center of each sample. Methods used for determination of pH and water activity (a_w) were adopted from Microbiological Laboratory Guidebook of USDA/ FSIS (Dey and Lattuada, 1998). For determination of pH, 10 g of *keropok lekor* were blended with 10 ml of distilled water. pH of the homogenized sample was measured with a pH-meter (Mettler Toledo). Water activity was measured with AquaLab water activity meter (Decagon Pullman, WA). Samples were cut to tiny discs in order to fill the surface of the cups, and the water activity was measured at 24°C. Reading for internal temperature, pH and water activity was taken as duplicates for each sampling.

Statistical analysis

All the data collected were analysed using the SAS 9.1 statistical package (SAS Institute, Inc. 2002-2003), using one-way analysis of variance (ANOVA). The Duncan's multiple range was used to determine significant differences among means. Thus, all the data reported were the means of five replicates.

Results

The total plate, psychrotrophic, yeasts and molds and mesophilic spore counts of raw minced fish were 6.95 ± 0.45; 4.72 ± 0.22; 2.67 ± 0.40; 1.97 ± 0.54 $\log_{10}$ cfu/g, (Figure 1), respectively. The *S. aureus* counts were 4.54 ± 0.46 $\log_{10}$ cfu/g, whereas, coliforms and faecal coliform counts were 4.04 and 3.83 log MPN/g (Figure 2), respectively.

The microbial profile of the samples, taken after mixing and kneading, did not show any significant difference ($p<0.05$) as compared to the minced fish. Total plate, psychrotrophic, yeast and mold, and mesophilic spore counts after mixing and kneading were 6.46 and 6.47; 4.50 and 4.41; 2.48 and 2.31;

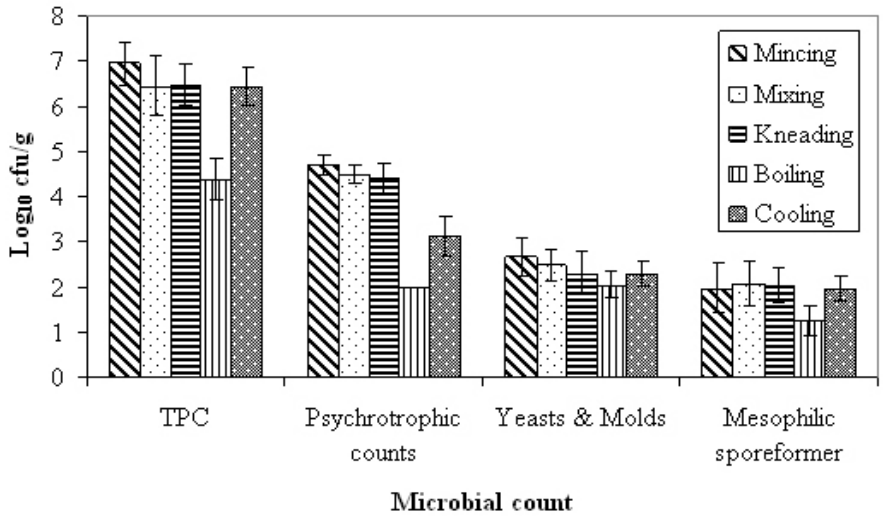

Figure 1. Total plate, psychrotrophic, yeasts and molds and mesophilic spore counts (log_{10} cfu/g) of *keropok lekor* during mincing, mixing, kneading, boiling and cooling stage [a]
[a] Means (SD from five determinations)

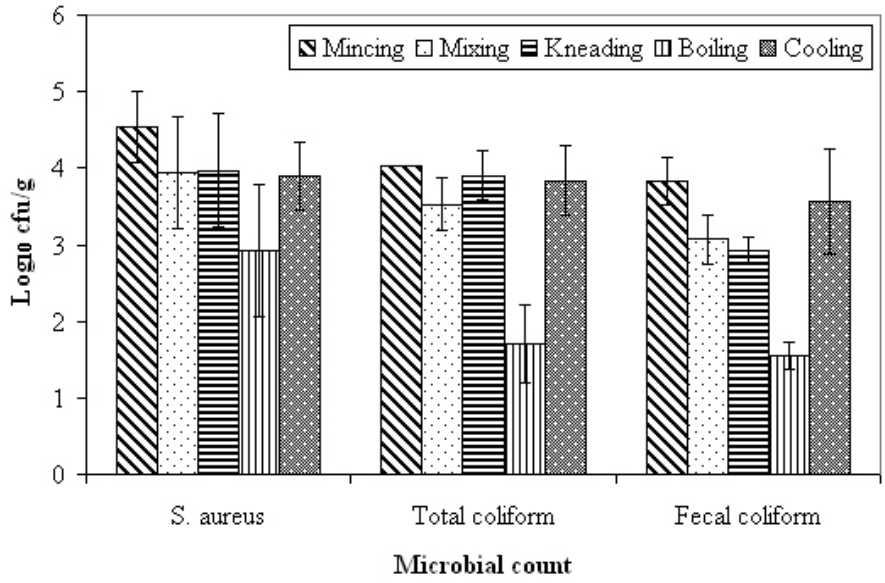

Figure 2. *S. aureus* counts (log_{10} cfu/g), coliforms and fecal coliform counts (log MPN/g) of *keropok lekor* during mincing, mixing, kneading, boiling and cooling stage[a]
[a] Means (SD from five determinations)

Table 1. Log reduction value of microbial counts after the boiling stage

Microbial profile	Log reduction value
Total plate count	2.09 log
Psychrophilic count	2.41 log
Yeasts and molds count	0.27 log
Mesophilic sporeformer count	0.78 log
S. aureus	1.05 log
Total coliform	2.19 log
Fecal coliform	1.38 log

Table 2. Microbial counts during kneading, boiling and cooling stage

Microbial profile	Processing stage		
	Kneading	Boiling	Cooling
Total plate count	6.47 ± 0.45[a]	4.38 ± 0.47[b]	6.44 ± 0.41[a]
Psychrophilic count	4.41 ± 0.34[a]	2.00 ± 0.00[c]	3.10 ± 0.44[b]
Yeasts and molds count	2.31 ± 0.45[a]	2.04 ± 0.28[a]	2.30 ± 0.26[a]
Mesophilic sporeformer count	2.04 ± 0.36[a]	1.26 ± 0.34[b]	1.97 ± 0.28[a]
S. aureus	3.97 ± 0.74[ab]	2.92 ± 0.87[b]	3.90 ± 0.45[ab]
Total coliform	3.90 ± 0.32[a]	1.71 ± 0.51[b]	3.84 ± 0.45[a]
Fecal coliform	2.93 ± 0.17[a]	1.55 ± 0.17[b]	3.56 ± 0.68[a]

[a-d] Means with different lowercase superscripts are significantly different ($p<0.05$) against row

Table 3. Presumptive of bacterial genus isolated at different stages of *keropok lekor* processing

Processing stage	n	Genus	Number of positive isolates (%)
Mincing	40	*Pseudomonas* spp.	17 (42.5)
		Micrococcus spp.	15 (37.5)
		Vibrio spp.	5 (12.5)
Mixing	40	*Micrococcus* spp.	19 (47.5)
		Bacillus spp	12 (30)
		Pseudomonas spp.	9 (22.5)
Kneading	60	*Staphylococcus* spp.	19 (31.7)
		Micrococcus spp.	17 (28.3)
		Enterobacteriaceae	14 (23.3)
		Pseudomonas spp.	7 (11.7)
		Vibrio spp.	3 (5)
Boiling	30	*Staphylococcus* spp.	17 (56.7)
		Bacillus spp.	13 (43.3)
Cooling	60	*Staphylococcus* spp.	17 (28.3)
		Micrococcus spp.	14 (23.3)
		Bacillus spp	12 (20)
		Aerococcus spp.	9 (15)
		Enterobacteriaceae	8 (13.3)

Total number of isolates 230 from PCA
n; pooled number of isolates from five sampling

2.07 and 2.04 $\log_{10}$ cfu/g, respectively. The counts for *S. aureus* after mixing and kneading were 3.94 and 3.97 $\log_{10}$ cfu/g, respectively, whereas coliform and fecal coliform counts were 3.53 and 3.90; 3.07 and 2.93 (log MPN/g), respectively.

The results indicated that the microbial counts had decreased significantly ($p<0.05$) after boiling as compared to after mincing, mixing and kneading stages (Table 1). *Keropok lekor* boiled at 100°C for 10 min reduced the TPC (4.38±0.47 $\log_{10}$ cfu/g), psychrotrophic counts (2.00 ± 0.00), mesophilic sporeformer counts (1.26 ± 0.34) and total coliform counts (1.71±0.51 log MPN/g) significantly ($p>0.05$). *S. aureus* (2.92±0.87 $\log_{10}$ cfu/g) and yeast and mold counts (2.04±0.28 $\log_{10}$ cfu/g) did not change significantly ($p<0.05$). After the cooling stage, the microbial counts for TPC, psychrotrophic, mesophilic sporeformer, total coliform and fecal coliform were found to increase significantly ($p<0.05$), except for the yeast and mold counts and *S. aureus* count (Table 2).

A total of 230 isolates from Plate Count Agar (PCA) plates were randomly selected and taken to represent the five different stages of *keropok lekor* processing (Table 3). 42.5, 22.5 and 11.7% of the isolates, belonging to the genus *Pseudomonas* spp.,

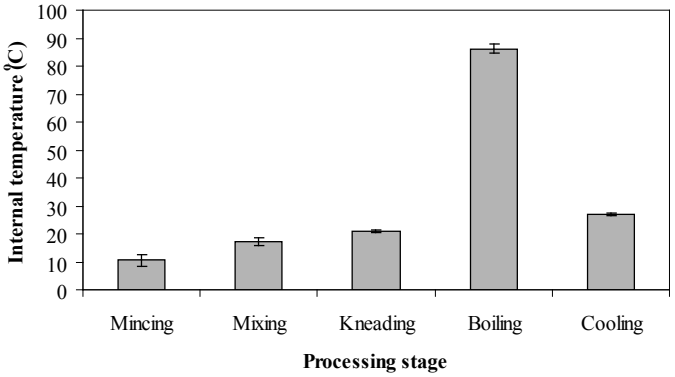

Figure 3. Internal temperature of *keropok lekor* during mincing, mixing, kneading, boiling and cooling stage [a]
[a] Means (SD from five determinations)

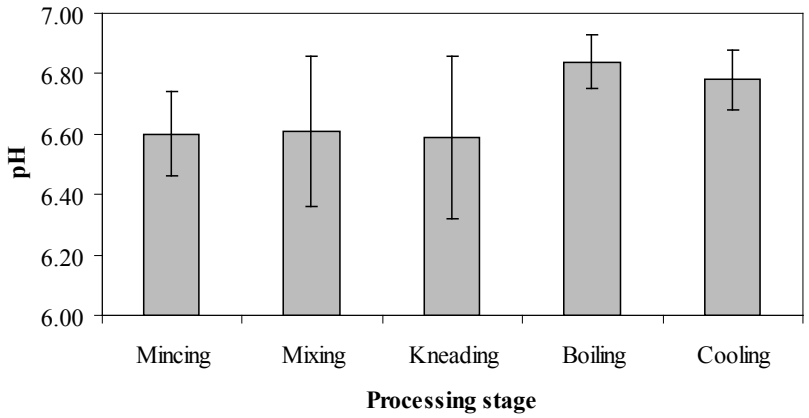

Figure 4. pH of *keropok lekor* during mincing, mixing, kneading, boiling and cooling stage [a]
[a] Means (SD from five determinations)

were isolated after the mincing, mixing and kneading stages, respectively. 37.5, 47.5 and 28.3% of the isolates, which were isolated after the mincing, mixing and kneading stages, belonged to the genus *Micrococcus*, respectively. After the boiling stage, it was found that *Micrococcus* spp. was not present. However, 23.3% of the isolates were indicated to belong to the genus *Micrococcus* after the cooling stage. 12.5 and 5% of the isolates, which were separated or isolated after the mincing and kneading stages, belonged to the genus *Vibrio*. 30, 43.3 and 20% of the isolates which were isolated after the mixing, boiling and cooling stages, belonged to genus *Bacillus*. Nevertheless, *Bacillus* spp. was not isolated during the kneading stage.

In this study, *Staphylococcus* spp. was predominant after the kneading (31.7%), boiling (56.7%) and cooling (28.3%) stages. In addition, *Enterobacteriaceae* spp. (23.3%) was isolated after the kneading stage, but not after the boiling. Meanwhile, 13.3% of the isolates isolated after the cooling stage were members of the *Enterobacteriaceae* family. Nonetheless, *Aerococcus* spp. (15%) was only found to be present during the cooling stage.

The internal temperature of *keropok lekor* during processing is shown in Figure 3. After mincing, mixing and kneading stages, the internal temperature was found to increase from 10.47 to 20.88°C. The internal temperature of the *keropok lekor* samples after the boiling stage was 86.27°C. After the cooling stage, the internal temperature of *keropok lekor* was indicated to decrease to 27.07°C.

The pH of the *keropok lekor* samples is presented in Figure 4 In this study, the pH of the *keropok lekor* samples did not change significantly ($p<0.05$) at the different stages of *keropok lekor* processing. The water activity (a_w) of *keropok lekor* during processing is presented in Figure 5. The water activity of *keropok lekor* did not change significantly ($p<0.05$) during the different stages of processing. This was found to range from 0.9831 to 0.9878.

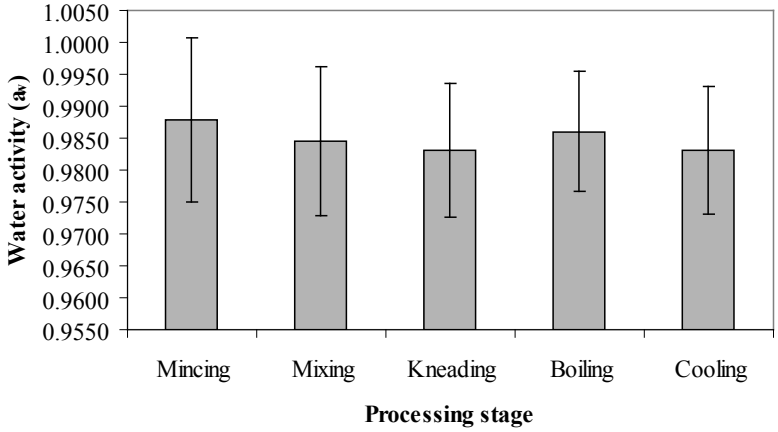

Figure 5. Water activity (a_w) of *keropok lekor* during mincing, mixing, kneading, boiling and cooling stage [a]
[a] Means (SD from five determinations)

Discussion

The growth conditions for microorganisms are due to specific intrinsic and extrinsic factors such as temperature, water activity, pH, oxidation-reduction potential (Eh), microbial interactions, nutrient content, etc. (Gram and Huss, 1996). *Keropok lekor* may provide an ideal condition for the microbial growth, with a suitable pH (pH 6 – 7) and water activity (aw 0.96 – 0.99). The microbial counts for the TPC in raw minced fish were high. This reflected the quality of the raw material which was used in the manufacturing. The initial quality of the raw materials is an important factor which influences the quality of the end product (Fuselli *et al*., 1994). The microbial counts for the TPC of minced fish used for *keropok lekor* making in the present study, were higher than the counts recorded in a study on the minced meats prepared for sausages (Sachindra *et al*., 2005) and exceeded the microbiological standards of fresh meat (ICMSF, 1980).

The dough of *keropok lekor* (after mixing) did not show any significant change in terms of microbial counts, but the counts of *S.aureus*, coliform and fecal coliform were found to decrease significantly from that of the minced fish. In contrast, Sachindra *et al*. (2005) observed no change of *S.aureus* and increase of coliform. Moreover, the microbial counts after kneading did not show any significant increase.

The internal temperatures measured in the samples were found suitable for an active growth of most of the microorganisms (Jay, 2000). Microorganisms require water and suitable pH medium for their growth; hence, limiting the amount of water content and altering the pH will affect the microbial multiplication in food (Forsythe and Hayes, 1998). The values of pH and water activity in the samples observed showed that ingredients used, such as salt and MSG and heat treatment applied, did not affect or impart significant changes in the pH and water activity. However, the range of pH and water activity in *keropok lekor* observed was indicated to be a supportive parameter of the high microbial counts in the samples of *keropok lekor*, as the ranges were found to favour the growth of most microorganisms in general (Jay, 2000).

Although microbial counts were high in the initial stages of processing, these increased counts did not have much relevance from the point of microbial safety, since the cooking process followed after that. The cooking process, by boiling at 100°C for 10 minutes in *keropok lekor* processing, was effective in reducing the microbiological counts. This was also observed by Borch *et al*. (1988) who found that the mesophilic counts were reduced in the product immediately after the heat processing. The microbial counts recorded in boiled *keropok lekor,* in the present study, are in agreement with the findings reported by Sachindra *et al*. (2005), and within the suggested microbiological standards for fish and fish products (ICMSF 1986). Thus, the results indicated that the heat treatment employed for the processing of *keropok lekor* was adequate from the aspects of microbiological quality and safety. Moreover, Borch *et al*. (1988) also observed that an effective heat processing was essential during the cooking in order to eliminate heat tolerance bacteria, so as to enhance the shelf life of product.

The application of heat to an internal temperature of 63ºC for 15 seconds for seafood during cooking, as recommended by FDA's 1997 Food Code, was reported as safe for consumption (Kurtzweil, 1999). However, this temperature is only sufficient for the destruction of vegetative form of pathogens, and

is therefore not the sole safety factor for the whole production process (Huss, 1997). Consequently, *keropok lekor* manufactured from the processing plant in this study could be referred to as a sort of semi-preserved food since the heat processing is not enough to kill all the microorganisms contained in the product. In other words, the product is pasteurized and not sterilized. Hence, the results of the current study corroborate the earlier study by Yokoseki (1957) who reported that fish sausage was not sterile and could contain up to 10^3 bacteria/g of sausage meat after the heat processing at 85 to 90ºC for approximately 1 hour.

The present study observed that the microbial count was increased significantly after the cooling process, i.e. up to 10^6 $\log_{10}$ cfu/g. This count is slightly higher than the finding of Tokur *et al*. (2006) and Al-Bulushi *et al*. (2005) who indicated that the total plate count in fish finger and fish burger was 10^5. The cooling process which was carried out at the room temperature had reduced the internal temperature of *keropok lekor* from 86.27 to 27.07°C. The reduction of the internal temperature made *keropok lekor* to be exposed to the danger zone, within 4 to 60°C. In other words, the condition of *keropok lekor* after cooling favours the growth of most microorganisms (Jay, 2000). According to Sachindra *et al*. (2005), the processing conditions such as the heat treatment might reduce microbial levels, but recontamination could take place during the post-processing handling or storage practice. The presence of high *S. aureus* and total coliform counts after the cooling stage might also be attributed to the recontamination when handling the boiled *keropok lekor*. As reported by Yokoseki (1957), even though fish sausage was heated, the product could not be considered as sterile. Besides, the microbial growth after the heat treatment is also influenced by the pH and a_w (Jay, 2000). The pH of *keropok lekor* was within pH 6 to 7 and a_w was within 0.97 to 0.99.Thus, these range of pH and a_w is suitable and can enhance the growth of microorganisms.

Bacterial species, which belong to 5 genera namely *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Aerococcus* spp. and *Enterobacteriaceae,* were isolated at the different stages of *keropok lekor* processing. These organisms have been associated with food handlers, equipment and raw materials (Ingham, 1991). Sanitary condition of workers and equipment has a very important influence on the microbiological quality of fish products. It is imperative that equipment was cleaned and sanitized often and that workers practice good hygiene. Only Gram positive bacteria were isolated after the boiling process; these were *Staphylococcus* spp. and *Bacillus* spp. This finding is in line with Raju *et al*. (2003) and Gram and Hush (1996) who reported that processed sausages provided an ideal medium for the growth of Gram positive bacteria. Another researcher (Amano, 1965) also reported that this maybe due to the raw fish used in the processing that might have been contaminated by the heat resistant Gram positive bacteria during landing and transportation. Furthermore, the ability to survive heat treatment can be attributed to carbohydrates and protein contents as well as the pH of *keropok lekor*. The presence of carbohydrates and protein has a protective effect on microorganisms and consequently increases the heat resistance of microorganisms (Jay, 2000). Moreover, microorganisms are most resistant to heat at their optimum pH of growth, which is generally about pH 7 (Jay, 2000).

The presence of *Staphylococcus aureus* indicates cross contamination or mishandling during processing (Ingham, 1991). This organism grows well in protein-rich foods and it is tolerant of high levels of salt. *S. aureus* is naturally present in the nose, throat, skin and hair (feathers) of healthy humans, animals and birds. This organism has also been indicated as present in wounds, boils and cuts in skin. *S. aureus* can also be isolated from feces and from a wide range of other environmental sites such as soil, marine and fresh water, plant surfaces, dust and air (Adam and Moss, 2000). The processing of *keropok lekor* involves lot of manual handling and this might be one of the sources of *Staphylococcus* spp. contamination.

In this study, the *Bacillus* species were also isolated after the boiling stage. The water activity, pH and temperature may play an important role in spore germination and growth of vegetative cells of *Bacillus* spp. (Condon *et al*., 1996; Quintavalla and Parolari, 1993). These aerobic, rod-shaped, spore-forming bacteria may probably be incorporated into the dough by flour contaminated with bacterial spores (Pattison *et al*., 2003). Spores which are not killed during boiling in a boiler subsequently germinate and grow. Furthermore, bacteria which survive the processing of food depend on pH, a_w and other micro-environmental factors (Condon *et al*., 1989). In addition, *Micrococcus* spp., *Aerococcus* spp. and *Enterobacteriaceae* were also present after the cooling stage, beside *Staphylococcus* spp. and *Bacillus* spp. Ingham (1991) stated that the extended periods, in which products were held at the microbial growth temperatures, must be avoided. This cooling process was done at the room temperature and held for a quite long duration that might enhance the growth of microorganisms.

The result of this study revealed variations in the distribution of the different microbial counts and

presence of different types of microorganisms at different stages of *keropok lekor* processing. Thus, reflect the level of contamination of the product at the different stages of processing, the raw material used and the improper handling of the product during and after the processing.

Conclusion

The work described in this paper reports the presence of different types of microorganism at the various stages of *keropok lekor* processing. Lower initial microbial levels of raw materials, effective heat treatment during boiling and proper cooling procedure of *keropok lekor* would improve the microbiological quality.

References

Adams M. R. and Moss M. O. 2008. Food Microbiology. 3rd edn. p. 252-257. Cambridge, UK: Royal Society of Chemistry.

Al-Bulushi, I. M, Kasapis, S., Al-Oufi, H. and Al-Mamari, S. 2005. Evaluating the quality and storage stability of fish burgers during frozen storage. Fisheries Science 71: 648-654.

Amano, K. 1965. Fish Sausage Manufacturing. In Borgstom, G. (Eds). Fish as Food, p. 265-280. New York: Academic Press.

APHA 2001. Downes, F. P. and Ito, K. (Eds). Compendium of methods for microbiological examination of foods. Washington, D.C.: American Public Health Association.

Borch, E., Nerbrink, E. and Svensson, P. 1988. Identification of major contamination sources during processing of emulsion sausage. International Journal of Food Microbiology, 7: 317-330.

Che Rohani, A. and Mat Arup, J. 1992. Some effects of sodium lactate on the quality of boiled fish sausage. Food Technology Research Station, MARDI. Report no. 11.

Condon, S., Lopez, P., Oria, R. and Sala, F.J. 1989. Thermal death determination: design and evaluation of a thermoresistometer. Journal of Food Science 54: 451-457.

Condon, S., Palop, A., Raso, J. and Sala F.J. 1996. Influence of the incubation temperature after heat treatment upon the estimated heat resistance value of spore of *Bacillus subtilis*. Letter in Applied Bacteriology 22: 149-152.

Dey, B. P. and Lattuada, C. P. 1998. Physical Examination of Meat and Poultry Products. In Microbiology Laboratory Guidebook. 3rd edn. Vol. 1 and 2. p. 2.1-2.8. United State: USDA/FSIS Microbiology Division.

Embong, M. @ Ishak, S., Rahim, M. A. A. and Shafie, N. (1990). The effect of various treatments on the dehydration of fish sausage. In Mohamed, S. and Osman, A. (Eds). Proceedings of the seminar on advances in food research III. p. 61-73. Kuala Lumpur, Malaysia: Impaque Publishing.

FDA 1997. Irradiation in the production, processing and handling of food. US Food and Drug Administration. Fed. Regist. 62, 64102–64121.

Forsythe, S. J. and Hayes, P. R. 1998. Food Hygiene, Microbiology and HACCP. p. 15-20. Gaithersburg, Maryland: Aspen Publishers Inc.

Fuselli, S. R., Casales, M. R., Fritz, R., and Yeannes, M. I. 1994. Microbiology of the marination process used in anchovy (*Encraulis anchoita*) production. Lebensmittel-Wissenschaft Und-Technologie, 27: 214–218.

Gerhardt P., Murray R. G. E., Costilow R. N., Nester E. W., Wood W. A. and Krieg N. R. 1981. Manual of methods for general bacteriology. Washington D.C: American Society for Microbiology.

Gram, L., and Huss, H. 1996. Microbiological spoilage of fish and fish products. International Journal of Food Microbiology 33: 589–595.

Harrigan, W. F., 1998. Laboratory Methods in Food Microbiology. p. 201–202, 395–396. San Diego, CA: Academic Press.

Holt J. G., Krieg N. R., Sueath P. H. A., Satley J. T. And Williams S. T. 1993. Bergey's Manual of Determinative Bacteriology. Baltimore: Williams and Wilkins.

Huss, H. H. 1997. Control of indigenous pathogenic bacteria in seafood. Food Control 8: 91–98.

ICMSF (International Commission on Microbiological Specifications for Foods), 1978. Indicator microorganisms. In Thatcher, F. S. and Clark, D. S. Microorganisms in Foods 1: Their Significance and Methods of Enumeration. *p. 3-14. Toronto: University of Toronto Press.*

ICMSF (International Commission on Microbiological Specifications for Foods), 1983. Microorganisms in foods. Their significance and methods of enumeration. *Toronto: University of Toronto Press.*

ICMSF (International Commission on Microbiological Specifications for Foods), 1980. Microbial Ecology of Foods. International Commission on Microbiological Specifications for Foods Vol. 2. New York: Academic Press.

ICMSF (International Commission on Microbiological Specifications for Foods), 1986. Microbiological Analysis: Principles and Specific Applications. p. 181–196. *Toronto: University of Toronto Press.*

Ingham, S. C., 1991. Microbiology of Mince, Surimi and Value-Added Seafoods. In Microbiology of Marine Food Products. p. 89-105. AVI Book.

Jay, J. M., 2000. Modern Food Microbiology. p. 35-41, 388-395. Gaithersburg, Maryland: Aspen Publishers, Inc.

Kurtzweil, P. 1999. Critical Steps Towards Safer Seafood. Washington DC, USFDA: FDA Consumer, Publication No. (FDA) 99-2317.

Kyaw, Z. Y., Yu, S. Y., Cheow, C. S. and Dzulkifly, M. H. 1999. Effect of steaming time on linear expension of fish crackers (Keropok). Journal Science Food Agricultural 79: 1340-1344.

Mossel, D. A. and Moreno Garcia, B. 1985. Técnicas para el análisis microbiológico de los alimentos. In: Microbiología de los Alimentos. p. 214–272. Zaragoza, España: Acribia, S.A.

Pattison, T. L., Lindsay, D. and Vonholy, A. 2003. In vitro growth response of bread-spoilage Bacillus strains to selected natural antimicrobials. Journal of Basic Microbiology 43: 341-347.

Quintavalla, S. & Parolari, G. 1993. Effect of temperature, a_w and pH on the growth of Bacillus cells and spores: a response surface methodology study. International Journal of Food Microbiology 19: 207-216.

Raju, C. V., Shamasundar, B. A. & Udupa, K. S. 2003. The use of nisin as a preservative in fish sausage stored at ambient (28±2°C) and refrigerated (6±2°C) temperature. International Journal of Food Science and Technology 38: 171-185.

Sachindra, N. M., Sakhare P. Z., Yashoda K. P. and Narasimha Rao D. 2005. Microbial profile of buffalo sausage during processing and storage. Food Control 16: 31–35.

SAS Institute Inc. 2002-2003. SAS User's Guide: Statistics. SAS Institute Inc.

Sasayama, S. 1973. Irradiation preservation of fish meat jelly products. II. Classification of spoilage bacteria in irradiated kamaboko. Bulletin Tokai Reg. Fish Research Laborotary 75: 39-46.

Thornley, A. 1960. The differentiation of Pseudomonas from other Gram negative bacteria on the basis of arginine metabolisme. Journal of Applied of Bacteriology 23: 37-52.

Tokur, B., Ozkutuk, S., Atici, E., Ozyurt, G. and Ozyurt, C. E. 2006. Chemical and sensory quality changes of fish fingers, made from mirror carp (*Cyprinus carpio* L., 1758), during frozen storage (-18°C). Food Chemistry 99: 335-341.

Yokoseki, M. 1957. Studies on the internal spoilage of fish jelly. I. Surviving microorganisms in fish jelly products cooked at different temperatures. Bulletin Japan Society for Scientific Fisheries 25: 581-588.