EFFECTS OF CHILLIES MARINADE ON THE MICRO-
BIOLOGICAL QUALITY OF INDIAN MACKEREL
(Rastrelliger kanagurta) AT CHILLED STORAGE

Karim Ulfah Nurul
Department of Fisheries Science, Faculty of Fisheries and Aqua Industry,
University Malaysia Terengganu (MALAYSIA)

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ABSTRACT
Mackerel fillet were vacuum-packed and subjected to chillies marinades as treated samples and left untreated as controls. Bacteria numbers and species diversities in all samples that were stored in iced storage at 4 °C for up to 16 days were studied. Results showed that numbers of total aerobic bacteria in treated mackerel were significantly (p<0.05) lower than in untreated mackerel by 2 log<sub>10</sub> CFU/g in all samplings. At the initial day, the bacterial counts in untreated samples increased gradually (p<0.001) from 5.82 log<sub>10</sub> CFU/g to reach 8.27 log<sub>10</sub> CFU/g after 16 days of chilled storage. Meanwhile a steady increased of bacteria counts in marinades mackerel showed from 4.36 log<sub>10</sub> CFU/g at 0 day to 7.37 log<sub>10</sub> CFU/g after 16 days of storage. Similar results were found in anaerobic and psychrotrophic bacteria count. Analysis of the bacterial flora revealed 4 different classes within the bacterial communities. The majority were Gram positive bacteria, with Bacillus sp. dominated by 33 %. This followed by Streptococcus, Staphylococcus and Lactobacillus, Clostridium or Actinomycetes by 17 % respectively in untreated samples. In addition, gram negative bacteria colonies representing 17 % in untreated samples. Marinating the mackerel eliminates the Gram negative bacteria. Treated samples were dominated by the gram positive bacteria presenting Coryneforms, Bacillus sp. and Micrococcus of 57 %, 29 % and 14 %, respectively. Raw Indian mackerel reach the limit of acceptability of 10<sup>7</sup> CFU/g at day 3 meanwhile the marinated samples extend the shelf-life up to 14 days of chilled storage.

Key Words: Microbiology quality, Indian mackerel, Chilled storage, Chilies marinades, Actinomycetes

INTRODUCTION
Food quality and safety assurance has become a major concern in many local industries as well as in the global trades. Food quality can be described in many perspectives depending on the product, processing handling and consumers. The food quality also can be determined by evaluating its appearance, flavor, odour, texture, nutritional value and its safety level. Fish quality can be defined as the overall characteristics and features of the fish or service that able to satisfy the stated or implied need (ISO 8402). The changes in fish and its products that are unacceptable or unsafe for human consumption are classified as the fish spoilage. A muscle of a living fish is in sterile state even some of microorganisms are exists in its skin’s slime, gills and alimentary tract. The defense mechanisms against the bacteriological infection in fish body will prevent the spoilage or the deterioration in a living fish. As soon as the fish dead, microorganisms will then penetrate into the fish flesh through the skins, belly cavity lining and gills. The deterioration will then starts to occur, where the microbiological activities, chemical reactions and autolytic processes combined together to form a deteriorative changes that lead to the fish spoilage. The enzymes that are secreted by the microorganisms especially from the specific spoilage bacteria such as Pseudomonas oraxella, Acinobacter, Alteromonas putrefaciens, Shewanella flavobacterium, Vibrio, Photobacterium and Aeromonas into the fish flesh were then react with the complex mixture of natural substances and forming the
deteriorative spoilage in fish. In addition, the handling processes such as storage temperature, rough handling, method of capture and mode of storage are also contribute in the fish spoilage. The preservation techniques are very important in seafood industry in order to prolong the shelf life of the raw fish and fish products. According to Kilinc and Cakli, marinade is a fish product that is processed by the treatments that gives a high characteristics flavour and able to retard the growth of bacteria but only in limited shelf life. The mix of spices that has been formulated in marinades has the abilities as the antimicrobial activities against the bacteria. Previous study from Cadun et al., reported that the marinated formulation for deep-water pink shrimps with rosemary extract is inhibited the microbial load after storage at 1°C. The effective of the semi preservative technique, which is chilled marinating in Indian mackerel with regards to the microbiological quality were studied.

**MATERIAL AND METHODS**

**Sample preparation**

The raw Indian mackerel were harvested commercially, gutted and transported from the local market to the laboratory in ice within 24 h of capture. Matched pairs of fillets were prepared, individually placed in polyethylene/polyamide bags and vacuum packed. The fish fillets were marinades to chillies paste (10 %, fish/paste, w/w) as treated samples and vacuum packed before stored in 4°C for 16 days.

**Microbiological analysis**

Microbiological qualities were analysed according to method described by Linton et al. Karim et al. with a minor modification of 0.1 % buffered peptone water used as diluents. The incubation conditions used for total aerobe counts were 30°C for 48 h for psychrotrophic counts 7°C for 10 days and for anaerobe counts the plates were incubated at 35°C for 48 h in an anaerobic jar (Oxoid AG25). Microbial analyses were carried out on day 0, 2, 4, 8, 12 and 16 for each sample treatments and for the corresponding control samples. The Colony-Forming Unit (CFU) of the bacteria were determined by choosing the plates that contained between 30-300 colonies of bacteria. The results of bacterial count were expressed as log_{10} CFU g^{-1} of the samples.

**Bacterial identification**

The identification of bacteria was carried out at the initial day and on day 12 which also corresponded approximately to the time when psychrotrophic counts stopped increasing in the controls. In all cases colonies were selected at random from the total aerobe, psychrotrophic and anaerobic plates using a Harrison Disc. The isolates were given a reference number, streaked onto PCA and incubated aerobically at 30°C. After 18-24 h incubation, the size and colour of the colonies were recorded and the following tests carried out Gram reaction, motility (using the hanging drop method), catalase and oxidase activities. The oxidation/fermentation test was carried out using Hugh and Leifson’s medium for Gram-negative bacteria or modified Baird Parker medium for Micrococc and Staphylococcus. If necessary, the isolates were stained for spore production using Schaeffer and Fulton’s modification of the Wirtz method. Bacteria were then placed in taxonomic groups according to the identification keys as described by Linton et al.

**Statistical analysis**

Analysis of variance (ANOVA) was used to search for significant differences between mean values of the different results. The results are presented as means ± SD.

**RESULTS AND DISCUSSION**

The total aerobic bacteria in marinade samples were significantly (p<0.05) lower than in untreated samples by 2 log_{10} CFU/g in all samplings (Fig. 1). At the initial day, the bacterial counts in untreated samples increased gradually (p<0.001) from 5.82 log_{10} CFU/g to reach 8.27 log_{10} CFU/g after 16 days of chilled storage.Meanwhile a steady increased of bacteria counts in marinades mackerel showed from 4.36 log_{10} CFU/g at 0 day to 7.37 log_{10} CFU/g after 16 days of storage (Fig. 1). The anaerobic bacteria in untreated Indian mackerel increased from 7.09 log_{10} CFU/g at 0 day to reach 7.73 log_{10} CFU/g after 16 days of chilled storage. Meanwhile anaerobic bacteria in marinade samples increased
to reach 7.52 log_{10} CFU/g after 16 days. However, there is no statistical different (p>0.05) of total anaerobic bacteria proved between the untreated and treated samples (Fig. 2). Similar results were found in psychrotrophic bacteria count (Fig. 3). At the initial day, the total psychrotrophic bacteria recorded at 4.98 log_{10} CFU/g and increased to maximum of 8.06 log_{10} CFU/g after 16 days of storage. Meanwhile, the counts were 4.58 log_{10} CFU/g in marinade samples raised to 7.84 log_{10} CFU/g at the end of storage period.

Analysis of the bacterial flora revealed 4 different classes within the bacterial communities. In the control samples, 83 % of gram-positive bacteria were observed and another 17 % were the gram-negative bacteria. The majority were gram positive bacteria, with
Bacillus sp. dominated by 33%. 16% were Streptococcus, 17% were Staphylococcus aureus and another 17% were Lactobacillus, Clostridium or Actinomycetes in untreated samples. In addition, Gram negative bacteria colonies representing 17% in untreated samples (Fig. 4). Marinating the mackerel eliminates the Gram negative bacteria. Treated samples were dominated by the Gram positive bacteria presenting Coryneforms, Bacillus sp. and Micrococcus of 57%, 29% and 14%, respectively (Fig. 5).

Based on the identified bacteria from this study, most bacterial colonies observed were the Gram-positive bacteria, such as Bacillus spp., Coryneforms, Staphylococcus sp., Micrococcus and Streptobacillus. Previous study from, the Gram-negative bacteria gives major contribution to the aerobic spoilage of marine fish rather than the Gram-positive bacteria but further handling
and processing of the fish can cause the Gram-positive bacteria to grow and cause the deteriorative spoilage. Even the marine fishes are predominated by the Gram-negative bacteria, but the interaction between the small percentage of Gram-positive bacteria with other groups of bacteria and its proteolytic activities at mesophilic temperatures (20-45 °C) can lead to the spoilage of the tropical marine fish.\textsuperscript{13,14}

**CONCLUSION**

Raw Indian mackerel reach the limit of acceptability of $10^7$ CFU/g at day 3 meanwhile the marinated samples extend the shelf-life up to 14 days of chilled storage. The obtained results also showed that the marinating effects have the abilities to inhibit the growth of Gram negative bacteria in Indian mackerel.

**REFERENCES**