UNIVERSITI PUTRA MALAYSIA

MICROBIOLOGICAL AND CHEMICAL QUALITY OF *KEROPOK LEKOR* DURING PROCESSING AND STORAGE

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By

NOR KHAIZURA BINTI MAHMUD @ AB. RASHID

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

March 2008

Specially dedicated to my soul mate: Ismail Fitry

my lil' caliph: Uzair Aqil

my lovely parents: mummy and ayah

for their constant prayer for my success

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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March 2008

Chairman : Associate Professor Zaiton Hassan, PhD

Faculty : Food Science and Technology

Keropok lekor is an important fish product in Malaysia. The customers' demands for *keropok lekor* have been increasing. This study was conducted to analyze the microbiological and chemical quality of *keropok lekor* in every stage of its processing, namely mincing, mixing, kneading, boiling and cooling. Subsequently, this study was also undertaken in an attempt to determine the effectiveness of post processing treatment on *keropok lekor* in order to prolong its shelf life. The method used to analyze the microbiological quality is known as the direct plate counts for the total plate counts (TPC), psychrotrophic, yeasts and molds, mesophilic sporeformer, *Staphylococcus aureus*, total coliform and fecal coliform counts. Simple biochemical test was carried out to identify the presumptive bacteria present in *keropok lekor* processing. Chemical quality was analyzed on the total volatile bases (TVB) and trimethylamine (TMA), using Conway microdiffusion method, and biogenic amines was done using the High Performance Liquid Chromatography (HPLC). The post-processing treatments on *keropok lekor* were exposing *keropok lekor* to UV light for 15 or 30 min, either coated with different concentrations of ascorbic acid (500, 1000 or 1500

ppm) or dipped in hot oil for 3, 6 or 9 s, and stored at the room temperature for 7 d or at chill temperature (4±1°C) for 14 d. When processing *keropok lekor*, the boiling of *keropok lekor* at 100°C for 10 min reduced the TPC (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 MPN/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 *S. 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 genus*. After the boiling stage, the presumptive predominant microorganisms were members of *Enterobacteriace* family and those belonging to *Micrococcus*, *Bacillus*, *Staphylococcus* and *Aerococcus genus*. As for the chemical quality, TVB and TMA levels were indicated to significantly decrease ($p<0/05$) after boiling from 7.29 to 4.68 mg/ 100g and 3.38 to 1.81 mg/ 100g, respectively, but not for the putrescine, cadaverine and histamine levels. Before the boiling stage, presumptive microorganisms producing putrescine, cadaverine and histamine were members of the *Enterobacteriaceae* family, as well as members of *Staphylococcus*, *Pseudomonas* and *Micrococcus* genus. Members of the genus *Pseudomonas,* which produce biogenic amines, were not isolated from *keropok lekor* after the boiling stage. The post-processing treatment which was applied on *keropok lekor* was found to enhance both its quality and shelf life. The results showed that exposing *keropok lekor* to UV light for 15 min and dipping it in hot oil for 9 s had extended the shelf life of this snack for 5 d when

stored at the room temperature, and for 14 d when stored at 4±1°C. This post processing treatment had also caused a significant reduction in TPC, psychrotrophic count, yeasts and molds count, TVB, as well as TMA and putrescine, cadaverine and histamine level. On the contrary, ascorbic acid was not as effective in increasing the shelf life of *keropok lekor* or in reducing TVB, TMA and putrescine, cadaverine and histamine level, as compared to dipping it in hot oil.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

KUALITI MIKROBIOLOGI DAN KIMIA BAGI KEROPOK LEKOR SEMASA PEMPROSESAN DAN PENYIMPANAN

Oleh

Nor Khaizura Binti Mahmud @ Ab. Rashid

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Keropok lekor merupakan produk hasilan ikan yang penting di Malaysia. Permintaan pengguna terhadap keropok lekor semakin meningkat. Kajian ini dijalankan untuk menganalisis kualiti keropok lekor dari aspek mikrobiologi dan kimia pada setiap peringkat dalam pemprosesan keropok lekor. Proses tersebut terdiri daripada mencincang isi ikan, menggaul, menguli, merebus dan menyejukkan keropok lekor. Seterusnya, kajian ini juga dilakukan untuk menentukan keberkesanan rawatan selepas pemprosesan ke atas keropok lekor dengan tujuan untuk memanjangkan jangka hayat produk. Kaedah yang digunakan untuk menganalisis kualiti mikrobiologi adalah pengiraan terus dari plat bagi total kiraan mikroorganisma (TPC), kiraan bakteria psychrotrophic, kiraan yis dan kulat, bakteria mesophilic yang menghasilkan spora, *Staphylococcus aureus*, total kiraan coliform dan fecal coliform. Ujian asas biokimia juga dijalankan untuk mengenalpasti bacteria yang mungkin hadir semasa pemprosesan keropok lekor. Kualiti kimia dianalysis melalui total volatile bases (TVB) dan trimethylamine (TMA) dengan kaedah Conway microdiffusion dan biogenic amine dengan kaedah komatografi cecair prestasi

tinggi (HPLC). Rawatan yang digunakan selepas pemprosesan dikenakan ke atas keropok lekor adalah dengan mendedahkan keropok lekor kepada cahaya UV selama 15 atau 30 minit dan seterusnya disalut dengan asid askorbik yang berkepekatan berbeza (500, 1000 atau 1500 ppm) atau dicelup ke dalam minyak panas selama 3, 6 atau 9 saat dan disimpan pada suhu bilik selama 7 hari atau suhu sejuk (4±1°C) selama 14 hari. Semasa pemprosesan keropok lekor, proses merebus keropok lekor pada 100°C untuk 10 min didapati dapat mengurangkan kiraan TPC (4.38±0.47 $\log_{10}$ cfu/g), kiraan bakteria psychrotrophic (2.00 ± 0.00), kiraan bakteria mesophilic yang menghasilkan spora (1.26 ± 0.34) and total kiraan coliform (1.71±0.51 log MPN/g) dengan signifikan ($p<0.05$). Namun demikian, kiraan mikroorganisma meningkat semula dengan signifikan ($p<0.05$) selepas proses menyejukkan keropok lekor kecuali kiraan yis dan kulat dan *S. aureus*. Mikroorganisma pradominan yang dapat dipencilkan sebelum proses merebus adalah daripada famili *Enterobacteriaceae* dan juga daripada genus *Pseudomonas, Vibrio*, *Staphylococcus*, *Bacillus* dan *Micrococcus*. Selepas proses merebus, mikroorganisma pradominan adalah daripada famili *Enterobacteriaceae* dan daripada genus *Micrococcus*, *Bacillus*, *Staphylococcus* dan *Aerococcus*. Bagi kualiti kimia, paras TVB dan TMA menunjukkan mengurangan yang signifikan ($p<0.05$) selepas proses merebus daripada 7.29 kepada 4.68 mg/ 100g dan 3.38 kepada 1.81 mg/ 100g, secara berturutan, tetapi tiada pengurangan yang signifikan bagi paras putrescine, cadaverine dan histamine. Mikroorganisma pradominan yang boleh menghasilkan putrescine, cadaverine dan histamine sebelum proses merebus didapati terdiri daripada famili *Enterobacteriaceae* dan juga daripada genus *Staphylococcus*, *Pseudomonas* dan

Micrococcus. Tiada genus *Pseudomonas* yang didapati boleh menghasilkan putrescine, cadaverine dan histamine dapat dipencilkan selepas proses merebus. Rawatan selepas pemprosesan yang dikenakan ke atas keropok lekor didapati dapat meningkatkan kualiti dan jangka hayat keropok lekor. Keputusan menunjukkan keropok lekor yang didedahkan kepada cahaya UV selama 15 minit dan dicelup ke dalam minyak panas selama 9 saat dapat memanjangkan jangka hayat keropok lekor kepada 5 hari apabila disimpan pada suhu bilik dan 14 hari apabila disimpan pada suhu 4±1°C. Rawatan selepas pemprosesan ini juga menunjukkan pengurangan secara signifikan pada TPC, kiraan bakteria psychrotrophic, kiraan yis dan kulat, paras TVB, TMA dan putrescine, cadaverine dan histamine. Asid askorbik didapati kurang berkesan dalam memanjangkan jangka hayat keropok lekor atau mengurangkan paras TVB, TMA dan juga putrescine, cadaverine dan histamine jika dibandingkan dengan mencelup keropok lekor dalam minyak panas.

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Finally, it is needless to say that my thesis is still far from being perfect even though I have made my best. Though it is so, I expect that this thesis contribute benefits to all readers and those who are involved in this.

I certify that an Examination Committee met on **date of viva** to conduct the final examination of Nor Khaizura Binti Mahmud @ Ab. Rashid on her Master of Science thesis entitled, "Microbiological and Chemical Quality of Keropok Lekor during Processing and Storage," in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree.

Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. Members of the Supervisory Committee were as follows:

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Date: 11 September 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

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NOR KHAIZURA BINTI MAHMUD @ AB. RASHID

Date:

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LIST OF ABBREVIATIONS

AA	Ascorbic acid
ANOVA	Analysis of Variance
a_w	water activity
g	Gram
HO	Hot oil
HPLC	High Performance Liquid Chromatography
ICMSF	International Commission on Microbiology Specifications for Foods
mg	Milligram
min	Minute
MPN	Most Probable Number
MSG	Monosodium glutamate
nm	Nanometer
ppm	Part per million
s	Second
TCA	Trichloroacetic Acid
TMA	Trimethylamine
TPC	Total Plate Count
TVB	Total Volatile Basic
UV	Ultraviolet

CHAPTER 1

GENERAL INTRODUCTION

The production of *keropok lekor* is one of the important traditional fish product industries in Malaysia. It is a popular snack food, not only in Malaysia but also in the Association of South East Asian Nations or ASEAN (Yu, 1992; Yeap and Tan, 2002). *Keropok lekor* is made from minced fish which is mixed with sago or tapioca flour. The processing of *keropok lekor* involves mainly five stages; these include mincing the fish meat, mixing the minced fish with other ingredients, kneading the dough, boiling and cooling before it is packed. This product can be easily found at night markets, hawker stalls and also most of the school canteens. *Keropok lekor* is usually served as an appetizer or a snack with special local-made chilli sauce.

The processing of *keropok lekor* is considered as labour-intensive, and this is usually carried by small and medium industries with little mechanization. The ingredients used in processing of *keropok lekor* are mostly according to the traditional recipes. However, the method used in its production has been improved, mainly with an addition of the machinery used in the processing. The mechanism in the processing is crucial in order to fulfil the increasing demand for *keropok lekor* in today's market. Nevertheless, a lot of manual handling is still widely practised in the processing of *keropok lekor*.