EFFECTS OF CURRY (Murraya koenigii Spreng.) LEAF EXTRACT ON THE MICROBIAL AND PHYSICO-CHEMICAL QUALITIES OF YELLOW ALKALINE NOODLES DURING STORAGE

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By

TEZA ALFINDO ROSYID

Thesis Submitted to the School of Graduate Studies,
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for the Degree of Master of Science

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Dedicate especially to…

I present this thesis to
My beloved mother Hj. Gustimaniar, SE, and father Prof. Ir. H. Rosyid, M.Sc
My brother and sister Garry Yuesa Rosyid, S.E, M.M and dr. Diah Fitriani Rosyid,
And my wife Norina Mohamad B.Sc
For their love, patience and understanding

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

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Chair : Associate Professor Roselina Karim, PhD

Faculty : Food Science and Technology

Yellow alkaline noodles (YAN) are very susceptible to spoilage due to the high pH and high moisture content of the product. The present study was conducted in an attempt to improve the shelf life of YAN using natural preservatives from local leaf extracts. This study involved the isolation and identification of the spoilage bacteria using the Biolog GEN III, screening of antibacterial activity of six types of local leaves on the identified bacteria followed by storage quality study of YAN treated with the most effective of leaf extracts.

In the first part of this study, eight bacteria were identified out of thirty colonies that were isolated. Five were Gram-positive (GP) bacteria which include *Bacillus pumilus,*

Clavibacter agropyri, Corynebacterium urealyticum, Corynebacterium jeikeium and *Staphylococcus sciuri,* and three were Gram-negative (GN) bacteria *Enterobacter cloacae, Pseudomonas aeruginosa* and *Serratia marcescens.* The *E. cloacae* and *S. sciuri* were found to be the most abundant bacteria on YAN with percentage of occurrence from all of thirty identified isolates at 23% and 26%, respectively. In the second part of the study, six local leaf extracts which were 'Pegaga' (*Centella asiatica*) 'Melur' (*Jasminum sambac*), 'Jarum tujuh bilah' (*Pereskia bleo*), 'Ulam raja' (*Cosmos caudatus*), 'Curry' (*Murraya koenigii*) and 'Tenggek burung' (*Melicope lunu*) were screened for their antibacterial activity against the identified bacteria in YAN. The agar disc-diffusion method was employed in the determination of antibacterial activities of organic and aqueous extracts. Generally, all the leaf extracts studied showed the presence of certain levels of antibacterial activity against identified bacteria in YAN. Leaf extracts of curry (*M. koenigii*) was discovered to possess effective antibacterial activity against GP and GN bacteria in YAN as compared to other leaf extracts. The observed inhibition zone diameter (IZD) of organic extracts was in the range of 6.90 to 20.63 mm, whereas for aqueous extracts was between 6.43 to 14.45 mm. Tetracycline which was used as positive control (standard antibiotic) showed the highest IZD against GP and GN bacteria tested at i.e. in the range of 17.0 to 25.67 mm. Result of the study showed that curry leaf extracts had the most effective antibacterial activity on the storage quality of YAN in an effort to extend the shelf life by using a natural preservative. Treated YAN were prepared by adding 0.5 % of organic leaf extracts of curry, while the untreated YAN (control) was prepared without the addition of leaf extracts.

The noodle samples were stored at ambient temperature (28±2°C) for six days and at chilled temperature (4±2°C) for six weeks. The microbiological qualities (total aerobic mesophilic bacteria, psychrophilic bacteria, yeast and mould count) physico-chemical properties (hardness, adhesiveness, springiness, cohesiveness, resilience, moisture content, pH value, and colour characteristic) of both samples were analysed. The leaf extracts of curry was found to significantly ($P<0.05$) reduced the initial load of total aerobic mesophilic bacteria, psychrophilic bacteria and yeast and mould count in noodle at ambient and chilled temperatures. The textural property of treated YAN was significantly ($P<0.05$) affected by the addition of curry leaf extracts. Significant ($P<0.05$) changes on moisture content and pH value were observed in the samples, with treated having a lower values as compared to the control YAN. In term of colour measurement, both of control and treated YAN showed a significant ($P<0.05$) reduction in colour parameters during storage at both temperatures. Shelf life of treated YAN at ambient and chilled temperatures were extended to 5 and 5 weeks, respectively, while, the shelf life of control YAN at ambient and chilled temperatures were 1.5 days and one week, respectively. This finding implied that bioactive compound of the curry leaf extracts can be used to extend the shelf life with combination of chilled condition further improves the storage stability of YAN. The combination of this preservation with chilled condition could further improve the YAN shelf life.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan Ijazah Master Sains

KESAN EKSTRAK DAUN KARI (*Murraya koenigii* Spreng.) KEATAS SIFAT MIKROBIAL DAN FIZIKO-KIMIA PADA MI KUNING BERALKALI SEMASA PENYIMPANAN

Oleh

TEZA ALFINDO ROSYID

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Pengerusi : Profesor Madya Roselina Karim, PhD

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Mee kuning beralkali (MKB) sangat mudah rosak disebabkan oleh pH dan kandungan air yang tinggi pada produk tersebut. Kajian ini dijalankan untuk meningkatkan jangka hayat pada MKB menggunakan pengawet semulajadi menggunakan ekstrak daun tempatan. Kajian ini melibatkan pengasingan bakteria perosak MKB, pengenalpastian bakteria tersebut menggunakan sistem Biolog GEN III, pemeriksaan aktiviti antibakteria dari enam jenis daun tempatan keatas bakteria yang telah dikenalpasti diikuti dengan kajian kualiti penyimpanan MKB dirawat dengan ekstrak daun yang paling efektif.

Pada bahagian pertama kajian, lapan bakteria telah dikenalpasti daripada tiga puluh koloni yang diasingkan. Lima daripadanya adalah bakteria Gram-positif (GP) iaitu *Bacillus pumilus, Clavibacter agropyri, Corynebacterium urealyticum, Corynebacterium jeikeium* dan *Staphylococcus sciuri,* manakala tiga adalah bakteria Gram-negatif (GN) iaitu *Enterobacter cloacae, Pseudomonas aeruginosa* dan *Serratia marcescens*. Bakteria yang paling banyak ditemui ialah *E. cloacae* dan *S. sciuri* pada MKB dengan peratusan kehadiran sebanyak masing-masing, 23% dan 26% daripada tiga puluh isolat yang dikenal pasti. Pada bahagian kedua kajian, aktiviti antibakteria pada enam daun ekstrak tempatan yang terdiri dari 'Pegaga' (*Centella asiatica*) 'Melur' (*Jasminum sambac*), 'Jarum tujuh bilah' (*Pereskia bleo*), 'Ulam raja' (*Cosmos caudatus*), 'Kari' (*Murraya koenigii*) and 'Tenggek burung' (*Melicope lunu*) diuji terhadap bakteria perosak yang telah dikenal pasti dalam MKB. Kaedah agar difusi cakera telah digunakan untuk menentukan aktiviti antibakteria dalam ekstrak organik dan air. Secara umumnya, semua ekstrak daun yang dikaji menunjukkan tahap aktiviti antibakteria tertentu apabila diuji pada bakteria perosak dalam MKB. Ekstrak daun kari didapati menunjukkan aktiviti antibakteria tertinggi terhadap semua bakteria GP dan GN didalam MKB berbanding dengan ekstrak daun lain. Pemerhatian daripada diameter zon perencatan (IZD) didalam ekstrak organik berada dalam julat 6.90 hingga 20.63 mm, manakala untuk ekstrak air ialah diantara 6.43 hingga 14.45 mm. Tetrasiklin yang digunakan sebagai kawalan positif (antibiotik standard) menunjukkan IZD tertinggi terhadap bakteria GP dan GN yang diuji dengan julat 17.0 hingga 25.67 mm. Hasil kajian menunjukkan bahawa ekstrak daun kari mempunyai aktiviti antibakteria yang paling berkesan kepada kualiti penyimpanan MKB dalam usaha untuk melanjutkan jangka hayat dengan menggunakan bahan pengawet semulajadi. MKB yang dirawat

disediakan dengan menambah 0.5% ekstrak organik kari, sementara MKB yang tidak dirawat (kawalan) disediakan tanpa penambahan ekstrak daun.

Sampel mi disimpan pada suhu bilik (28±2°C) selama enam hari dan suhu sejuk (4±2°C) selama enam minggu. Kualiti mikrobiologi (jumlah bacteria aerobik mesofilik, dan bakteria psikrofilik, yis dan kulat) sifat fiziko-kimia (kekerasan, kelekatan, kekenyalan, kelekitan, ketahanan, kandungan air, nilai pH dan ciri-ciri warna) kedua-dua sampel dianalsis. Ekstrak daun kari didapati secara signifikan ($P<0.05$) mengurangkan jumlah beban awal dari jumlah keseluruhan bakteria aerobik, mesofilik, psikrofilik, pada yis dan kulat pada suhu bilik dan suhu sejuk. Sifat tekstur pada MKB yang dirawat berpengaruh signifikan ($P<0.05$) oleh penambahan ekstrak kari. Perubahan yang signifikan ($P<0.05$) dapat dilihat terhadap kadar air dan nilai pH sampel, dimana MKB yang dirawat memiliki nilai yang lebih rendah berbanding dengan MKB kawalan. Dari aspek pengukuran warna, kedua-dua MBK kawalan dan rawatan menunjukkan pengurangan yang signifikan ($P<0.05$) pada parameter warna sewaktu penyimpanan pada kedua-dua suhu. Jangka hayat MKB terawat pada suhu bilik dan suhu sejuk adalah masing-masing, 5 hari dan 5 minggu, sementara jangka hayat MKB kawalan pada suhu bilik dan suhu sejuk adalah masing-masing 1.5 hari dan satu minggu. Penemuan ini tersirat bahawa sebatian bioaktif ekstrak daun kari boleh digunakan untuk melanjutkan jangka hayat dengan kombinasi suhu sejuk dan meningkatkan lagi kestabilan penyimpanan MKB. Gabungan pengekalan ini dengan keadaan sejuk boleh meningkatkan lagi jangka hayat pada MKB.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figures **Page**

LIST OF ABBREVIATIONS

a_w	Water activity
APC	Aerobic plate count
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
DMSO	Dimethyl sulfoxide
DRBC	Dichloran Rose Bengal
EDTA	Ethylenediaminetetraacetic
EtOH	Ethanol
GP	Gram-positive
GN	Gram-negative
g	Gram
H_2O	Water
IZD	Inhibition zone diameter
IF	Inoculating fluid
K_2CO_3	Potassium carbonate
MMEs	Monolaurin microemulsion system
MHA	Mueller-Hinton agar
MKB	Mi kuning beralkali
mm	Millimetre
mg	Milligram
ml	Millilitre

NA	Nutrient agar
NCCLS	National Committee Clinical Laboratory Standard
NaCl	Sodium chloride
Na_2CO_3	Sodium carbonate
PCA	Plate count agar
RH	Relative humidity
SPC	Standard plate count
SMI	Small and medium industries
TPA	Texture profile analysis
UV	Ultraviolet
WHO	World Health Organization
YAN	Yellow alkaline noodle
^0C	Degree Celsius
µl	Micro litre
µg	Micro gram

CHAPTER 1

INTRODUCTION

Noodles are strips cut from a sheet of dough. The dough is made from wheat flour, water, common salt (sodium chloride), and/or alkaline salt such as sodium carbonate (Miskelly, 1996). This dough is compressed between a series of rolls to form a dough sheet, and the sheeted dough is then slit to produce noodles. The gluten network developed during the sheeting process contributes to the noodles texture (Hou and Kurk, 1998).

Noodles are popular in many parts of Asia. It is one of the staple foods in the Orient (Wu *et al.,* 1998). There are many types of noodles consumed by people in South East Asia, China and Japan. They are generally classified in various ways such as, based on the raw materials, processing, size and shape of the end product and types of salt used (Miskelly, 1996; Hou and Kurk, 1998). The common types of noodles in Asia are white salted noodles (e.g. Japanese *udon*), yellow alkaline noodles (e.g. Chinese *hokkien*) and instant noodles (e.g. Korean *ramyon)* (Miskelly, 1998).

Yellow alkaline noodles (YAN) are one of the popular foods in Malaysia, Indonesia, and Singapore. It is made from wheat flour containing 11-12.5 % protein, water, salt and alkaline salt (Miskelly, 1996; Hou and Kurk, 1998). The addition of alkaline salt confers a unique flavour and quality, which is absent in pasta or other type of noodles. It also produces noodles with a pH range of 9.0 to 10.0. Boiled yellow

alkaline noodles generally have moisture content of 50-60 % (Karim, 1989) and hence, are very susceptible to spoilage and have a very short shelf life approximately about 1-1.5 days.

In order to extend the shelf life, some of manufacturers use chemicals such as boric acid, aflatoxins and formalin into the food products that can cause poisoning. Since no permitted preservative is currently available, boric acid which is banned by the Malaysian Food Act 1985 is still widespread use as a preservative in YAN. However, Yiu and See (2008) in their study had found that the concentration of boric acid was highest in five types of noodles and fish ball. In line with that, the Malaysian Ministry of Health in early March 2006 had stated the yellow noodle contained 70.4%, followed by wantan noodle with 14.8% of boric acid, and koay teow, laksa, loh see fun and spring roll with 3.7% of boric acid (The Star, 2006). Meanwhile, on June 15, 2007, Consumers Association in Penang (CAP) reported that rice dumplings collected from Penang, Ipoh, Taiping, K. Lumpur and Klang contained boric ranging from 0.4 to 479.6 ppm. Base on the report, that the local food manufacturers were still using boric acid although it is not a permitted food preservative in Malaysia. In view of the widespread misused of boric acid in the country, surveys on the boric acid levels used in locally manufactured food are considered necessary. Since, consumers are currently aware of the health benefits and risks associated with consumption of foods including chemical safety of food, more effort should be taken in studying alternative methods of food preservation.

Many researchers have reported the application of additives free methods to extend the shelf life of fresh pasta and noodles products. McGuire *et al.* (1989) applied hurdle technology using combination of dough pasteurization, modified atmosphere packaging, and chilling to preserve fresh pasta and succeeded in extending the shelf life to 120 days. Jianming (1998) discovered that irradiation by 10 kGy ^{60}Co-γ rays could increase the shelf life of fresh noodles up to 10 days when stored at room temperature. Fu (2008) succeeded in extending the shelf life of fresh noodles with neutral pH up to 10 days at 37°C using food grade Monolaurin Microemulsion system (MMEs). Other researchers have used the organic acid solution combined with heat treatment (i.e. pasteurization) to increase the shelf life of fresh pasta and noodles (Nagao, 1996; Wu *et al.,* 1998; O'Rourke *et al.,* 2003). Suitable conventional organic acids that have been used include citric, acid, acetic acid, tartaric acid, lactic acid, ascorbic acid, propionic acid and erythrobic acid (Howard *et al.,* 1994). Saito (2003) had reported on the usage of biological method in extending the shelf life which involved addition of *Brevibacterium helvolum* B8 and *Arthrobacter* sp. *B25* and he succeeded in improving the quality especially the colour and shelf life of alkaline noodles up to seven days.

Many new natural food preservation methods have been investigated by researchers. Del Nobile *et al.* (2009) discovered that natural antibacterial compounds of lemon extracts were effective in preventing microbial growth and improving the microbiological stability of refrigerated amaranth-based homemade fresh pasta. The presence of lemon extracts delayed the growth of mesophilic and psychrophilic bacteria. Zainol (2004) had demonstrated the utilization of highest flavonoid compounds of *Centella asiatica* when applied into herbal noodle.

Ningappa *et al.* (2010) demonstrated that monomeric protein with molecular mass 35 kDa isolated from curry (*Murraya koenigii*) leaf was found to posses potential antibacterial activity against all human pathogenic bacteria including *Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Klebsiella pneumoniae*, *Salmonella typhi* and *Bacillus subtilis*. They had proven that the leaf could effectively inhibited *E. coli, S. aureus* and *S. typhi.*

Although the antibacterial activity of components from traditional herbs spices has been well reported, there are still many considerable opportunities to identify and evaluate antibacterial properties of other native plants that may be potentially beneficial as food preservatives. Potentially useful antimicrobial plant compounds or extracts should ideally display inhibition activity against a wide range of microorganisms as foods are seldom contaminated by a single species (Gram *et al.*, 2002).

This study was carried out in an attempt to identify bacteria that causes spoilage in YAN, to find the most effective leaves extracts that could inhibit most of the spoilage bacteria in YAN, and to evaluate the effect of the most effective leaf extract on stability of YAN. At present, not much work has been reported on the application or usage of local leaves extracts in extending the shelf life of yellow alkaline noodles. Therefore, the effect of leaf extracts on the physico-chemical and microbial quality of yellow alkaline noodles were examined.

Based on the above discussion, the main objectives of this research were:

1. To isolate and identify the predominant spoilage bacteria of yellow alkaline noodles
2. To screen the antibacterial activity of several local leaves extracts against the identified bacteria in yellow alkaline noodles
3. To evaluate the effects of curry (*Murraya koenigii*) leaf extracts on the microbiological and physico-chemical qualities of yellow alkaline noodles during storage.

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