

EVALUATION OF SPONTANEOUS AND CONTROLLED
FERMENTATION USING DIFFERENT STARTER CULTURES
FOR LACTIC ACID FERMENTATION OF CIBBANGI

Shahidah Md. Nor
(Matric. No. 3120155)

Thesis Submitted in Fulfillment for the Degree of
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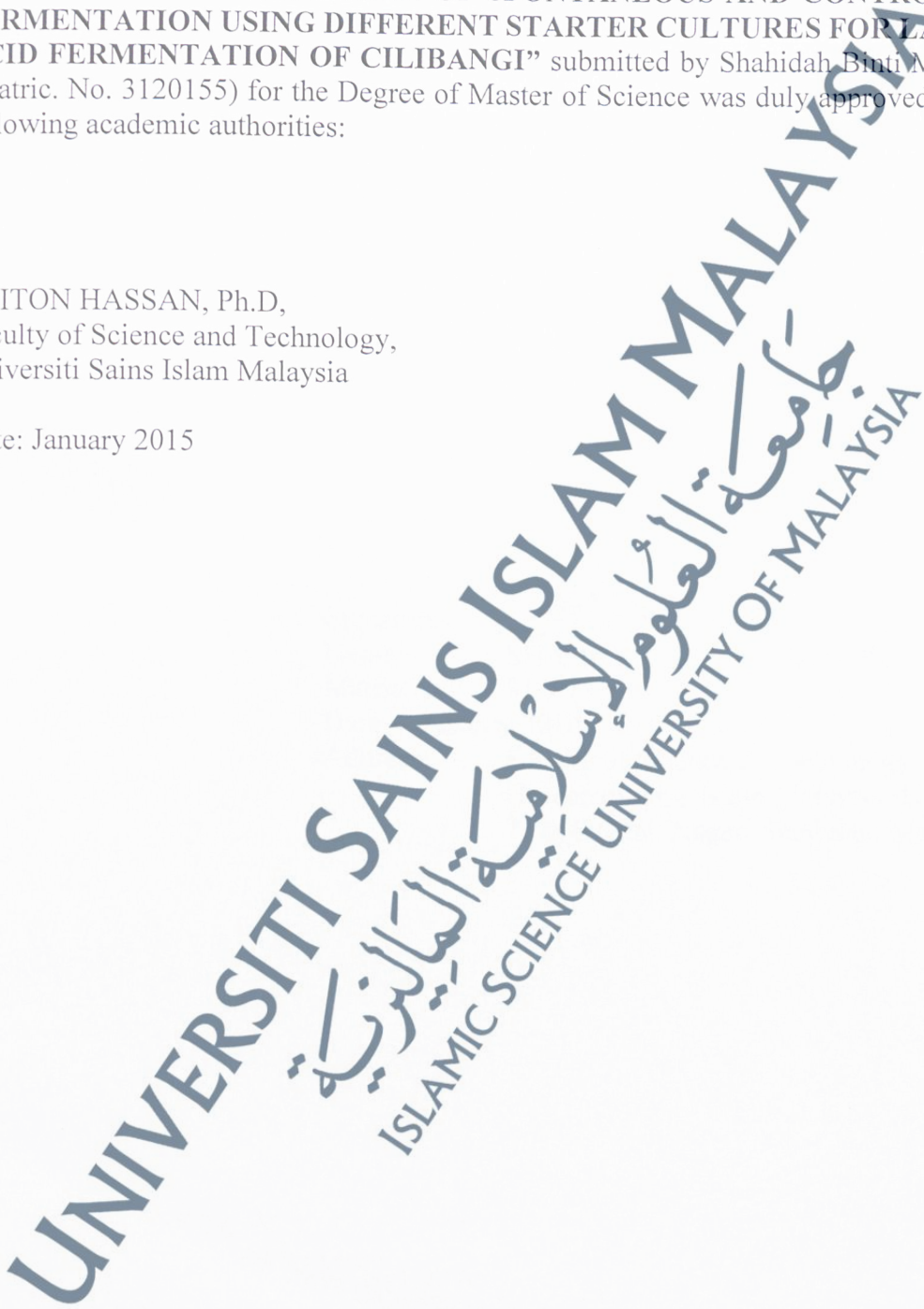
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APPROVAL

The thesis entitled “**EVALUATION OF SPONTANEOUS AND CONTROLLED FERMENTATION USING DIFFERENT STARTER CULTURES FOR LACTIC ACID FERMENTATION OF CILIBANGI**” submitted by Shahidah Binti Md. Nor (Matric. No. 3120155) for the Degree of Master of Science was duly approved by the following academic authorities:

ZAITON HASSAN, Ph.D,
Faculty of Science and Technology,
Universiti Sains Islam Malaysia

Date: January 2015



AUTHOR'S DECLARATION

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

I hereby declare that the work in this thesis entitled "EVALUATION OF SPONTANEOUS AND CONTROLLED FERMENTATION USING DIFFERENT STARTER CULTURES FOR LACTIC ACID FERMENTATION OF CILIBANGI" and submitted for the Degree of Master in Science my own except for quotations and summaries which have been duly acknowledged.

Signature: 

Name: SHAHIDAH BINTI MD. NOR

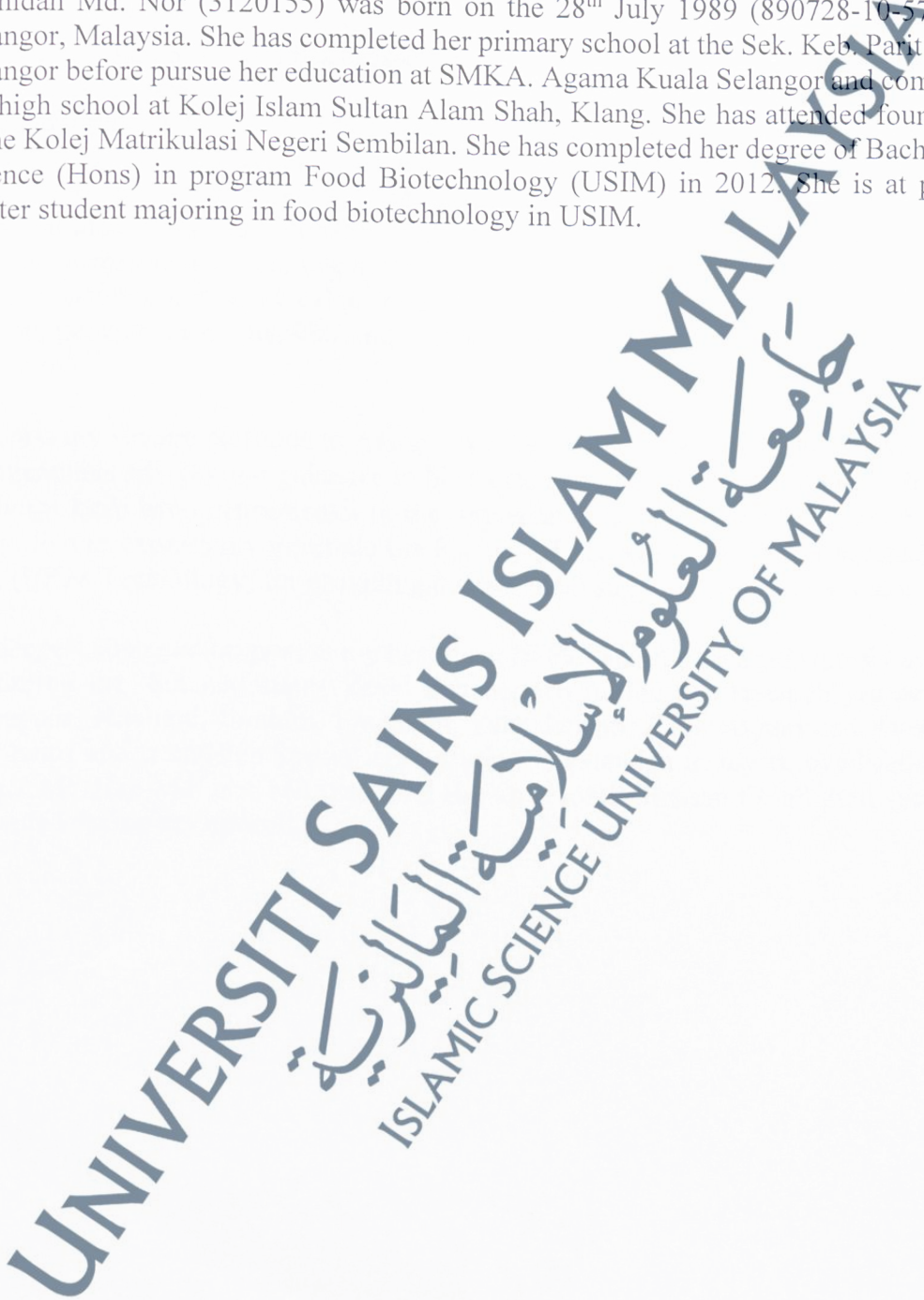
Matric No: 3120155

Date: 1 January 2016

Address: Faculty of Science & Technology
Universiti Sains Islam Malaysia (USIM)
71 800 Nilai, Negeri Sembilan, Malaysia

BIODATA OF AUTHOR

Shahidah Md. Nor (3120155) was born on the 28th July 1989 (890728-10-5786) in Selangor, Malaysia. She has completed her primary school at the Sek. Keb. Parit Enam, Selangor before pursue her education at SMKA. Agama Kuala Selangor and completed her high school at Kolej Islam Sultan Alam Shah, Klang. She has attended foundation at the Kolej Matrikulasi Negeri Sembilan. She has completed her degree of Bachelor of Science (Hons) in program Food Biotechnology (USIM) in 2012. She is at present master student majoring in food biotechnology in USIM.



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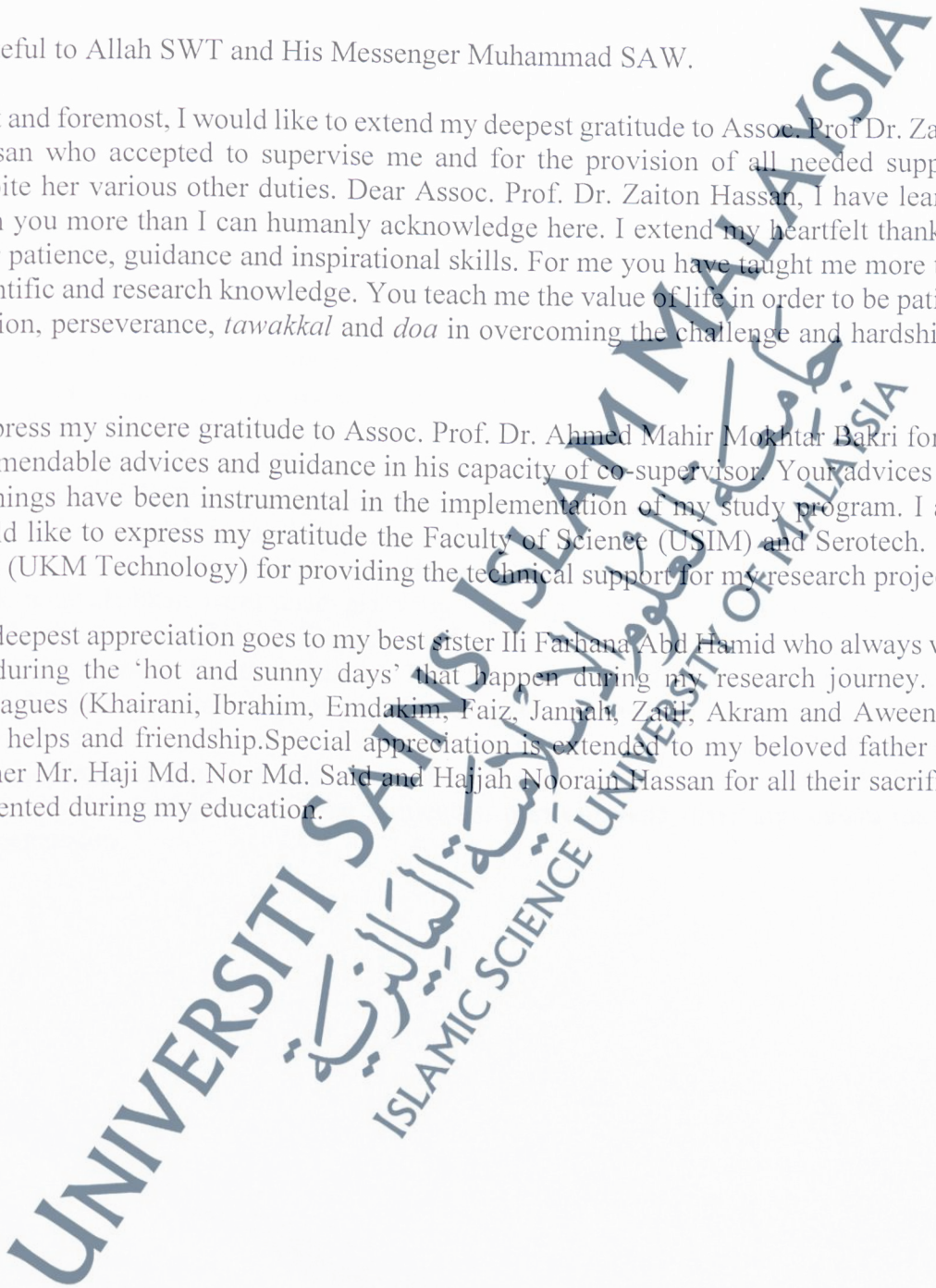
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Grateful to Allah SWT and His Messenger Muhammad SAW.

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ABSTRAK

Fermentasi puri cili yang disediakan secara tradisional oleh fermentasi spontan memerlukan kira-kira 1-48 bulan. Kajian ini telah dijalankan untuk mengenalpasti dan mencirikan bakteria asid laktik daripada dua sumber isolat; autochthonous (isolat daripada pra-fermentasi puri cili) dan allochthonous (isolat susu mentah, susu fermentasi buatan dan susu fermentasi komersial) berdasarkan sifat bau ujian penerimaan menggunakan analisis kuantitatif deskriptif (QDA) dan ujian hedonik pada fermentasi puri cili. Ujian QDA mendapati bahawa panel suka fermentasi puri cili yang menghasilkan bau yang masam, manis, bunga dan buah yang kuat. Cili sama ada boleh dipra-pasteurkan pada 80°C selama 15 min atau tidak dipra-pasteurkan sebelum penginokulasian bakteria asid laktik. Tiga daripada enam bakteria asid laktik telah menunjukkan sifat bau yang disukai dan telah dikenalpasti sebagai *Lactobacillus plantarum* AU2, *L. plantarum* ALO1, dan *L. pentosus* ALO2 menggunakan kaedah API 50CHL dan PCR 16S rDNA. Ketiga bakteria asid laktik ini telah dinilai bagi kemampuan untuk hidup, perubahan dalam pH dan asid titrasi selama 28 hari penapaian. Semua LAB hidup dengan baik dalam kedua-dua puri cili pasteuras dan tidak pasteuras (10^6 CFU/mL selepas 28 hari penapaian). Pra-pempasteuran puri cili merendahkan jumlah kulat dan yis, manakala inokulasi dengan *L. plantarum* ALO1 dan *L. pentosus* ALO2 menghalang pertumbuhan kulat dan yis. Inokulasi bakteria asid laktik menyebabkan penurunan pH yang cepat ($p < 0.05$) dan meningkatkan jumlah laktik asid selepas 7 hari fermentasi dalam FCM seperti dibandingkan kepada fermentasi spontan. Secara kualitatif, komponen organik meruap (VOC) dipengaruhi oleh LAB dan etanol adalah komponen yang paling banyak terdapat dalam fermentasi puri cili seperti yang telah dikesan oleh ruang udara statik GC-MS. Secara kesimpulannya, penginokulasian *L. pentosus* ALO2 telah mempercepatkan proses fermentasi dan menghasilkan bau fermentasi puri cili yang disenangi dalam masa 7 hari penapaian.

ABSTRACT

Fermented chili mash is traditionally prepared by spontaneous fermentation which takes about 1-48 month. This study was conducted to identify and characterize LAB from two sources; autochthonous (isolated from two months pre-fermented chili mash) and allochthonous (isolated from raw milk and home-made yoghurt) for their odor characteristics and acceptability using quantitative descriptive analysis (QDA) and hedonic test on fermented chili mash. QDA test revealed that the panelists preferred fermented chili mash that produced intense sourness, sweet, flowery and fruity smell. The chilies were either pre-pasteurized at 80°C for 15 min or non-pasteurized prior to LAB inoculation. Three from six LAB isolates showed desirable odor characteristics and were identified as *Lactobacillus plantarum* AU2, *L. plantarum* ALO1 and *L. pentosus* ALO2 using API 50CHL and PCR 16S rDNA methods. These three LABs were further evaluated for their survival, changes in pH and titratable acidity during 28 days fermentation of fermented chili mash at 35°C. All LABs survived well in both pre-pasteurized and non-pasteurized fermented chili mash (10^6 CFU/mL) after 28 days fermentation. Pre-pasteurization of fermented chili mash decreased the mold and yeast count, while inoculation with *L. plantarum* ALO1 and *L. pentosus* ALO2 inhibited the growth of mold and yeast. Inoculation of LAB caused significantly rapid reduction in pH ($p < 0.05$) and increased in lactic acid content after 7 days fermentation of fermented chili mash as compared to spontaneous fermentation. Qualitatively, volatile organic compound (VOC) is dependent on LAB strains inoculated and ethanol was the most abundant compound present in fermented chili mash as detected by static head-space GCMS. In conclusion, inoculation of *L. pentosus* ALO2 was able to accelerate the fermentation process and produced acceptable odor characteristics of fermented chili mash as early as 7 days of fermentation.

الملخص

هريسة الفلفل المتخمرة يتم تحضيرها تقليديا بطريقة التخمير الطبيعية والتي تأخذ حوالي من شهر واحد الى ثمانية واربعون شهرا. في هذه الدراسة قد اجريت تأثير بكتيريا حمض الاكتيك كبادئ وذلك لزيادة سرعة تخمير الهريسة حيث تم عزل بكتيريا حمض الاكتيك من مصادر مختلفة (الهريسة المتخمرة طبيعيا لمدة شهرين اللبن الخام والزبادي المصنع في البيت). وقد تم تقييم خصائص رائحة بالاحساس بالرضى للهريسة المتخمرة . وقد اظهر اختبار و(QDA) والقبول لهريسة الفلفل باستخدام التحليل النوعي وصفى المحكمين يفضلوا الهريسة التي بها نسبة عالية من الحموضة والحلاوة ورائحة الفواكهة ان(QDA) وفي هذه الدراسة تم تلقيح الفلفل المبسترة على درجة 80 درجة مئوية لمد 15 دقيقة او الفلفل الغير مبستر ببكتيريا حمض الاكتيك عن وقد اظهرت الدراسة بان الفلفل المبستر قبل عملية التلقيح بواسطة بكتيريا حمض الاكتيك لها تأثير معنوي ($p < 0.05$) بانخفاض في مستوى الحموضة ولكن ليس لها تأثير يذكر على الرائحة. و اظهرت ثلاثة من ستة عزلات من بكتيريا حمض الاكتيك صفات رائحة مرغوب بها وقد عرفت هذه العزلات بأختبار تخمر الكربوهيدرات واختبار الحمض النووي على انها *L. pentosus* ALO2 *L. plantarum* AU2 *L. plantarum* ALO1 وقد اجريت على هذه الثلاث عزلات دراسات اضافية على قدرة تحمل التغيرات في درجات الحموضة واختبار(تي اي) خلال فترة تخمير لمدة 28 يوما للفلفل المتخمرة على درجة حرارة 35 درجة مئوية. وقد اظهرت ان ثلاث عزلات من بكتيريا حمض الاكتيك ان لها القدرة على التحمل لكل من نوعين الفلفل المبستر والغير مبستر طيلة فترة التخمير والتي كانت 28 يوم. وفي فترة التخمير هذه لوحظ انخفاض في عدد الفطريات والخمائر للفلفل المبستر بينما عند التلقيح ببكتيريا حمض الاكتيك نوع (الو1 والو 2) أدت الى تثبيط نمو الفطريات والخمائر وكذلك تحضين بكتيريا حمض الاكتيك ادا الى انخفاض سريع في درجة الحموضة وزيادة في مستوى حمض الاكتيك بعد 7 ايام من تخمير لهريسة الفلفل المبسترة عند مقارنتها بي هريسة الفلفل المتخمرة بطريقة الطبيعية وبطريقة التحليل النوعي اتضح ان الايثانول كان هو الاكثر وفرة في هريسة الفلفل وذلك بطريقة الفراغ الرانسيز و في الختام تلقيح هريسة الفلفل ببكتيريا حمض الاكتيك نوع لاكتيك باسلاس بلانتروم الو1 و لاكتيك اسيد بنتوسيز الو2 لها القدرة على تسريع عملية التخمير وانتجت خصائص رائحة مقبولة.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	degree celsius
µl	microliter
µm	micrometer
bp	base pair
CaCO ₃	calcium carbonate
CFU	colony-forming unit
cm	centimeter
CPPs	calcium-binding phosphopeptides
d	day
dH ₂ O	distilled water
ddH ₂ O	double-distilled water
DNA	deoxyribonucleic acid
FT	fermentation time
HT	heating treatment
g	gram
h	hour
H ₂ O	water
LAB	lactic acid bacteria
m	month
M	mole
mg	milligram
min	minute
ml	milliliter
mm	millimeter
mM	millimole
MRS	Man, Rogosa and Sharpes
NA	nutrient agar
Na ₂ CO ₃	sodium carbonate
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram
nm	nano meter
np	non-pasteurize
p	pasteurize
PBS	phosphate-buffer saline
PCR	polymerase chain reaction
ppm	part per million
RNA	ribonucleic acid
RT	retention time
sec	second
TA	titratable acidity
TPC	total plate count
u	unit
UV	ultraviolet
v/v	volume/volume

w/v
 μg
VOC

weight/volume
microgram
volatile organic compound

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