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และการเปลี่ยนแปลงทางเคมีระหว่างกระบวนการหมัก



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**AUTOCHTHONOUS YEASTS ASSOCIATED WITH PINEAPPLE WINE
FERMENTATION AND CHEMICAL CHANGES
DURING FERMENTATION PROCESS**

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**A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Food Technology**

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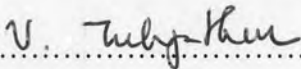
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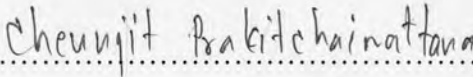
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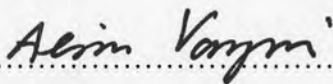
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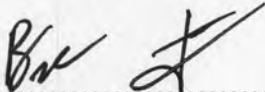

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อรอง จันทรประสาธุช: ออโตโคเนสซิสต์ที่เกี่ยวข้องกับการหมักไวน์สับปะรดและการเปลี่ยนแปลงทางเคมีระหว่างกระบวนการหมัก. (AUTOCHTHONOUS YEASTS ASSOCIATED WITH PINEAPPLE WINE FERMENTATION AND CHEMICAL CHANGES DURING FERMENTATION PROCESS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.รมณี สงวนดีกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ.ดร.ชินจิต ประกิตชัยวัฒนา, PROF. GRAHAM H. FLEET, Ph.D., 282 หน้า.

งานวิจัยนี้ได้ติดตามยีสต์ที่เกี่ยวข้องกับการหมักที่เกิดขึ้นเองตามธรรมชาติของน้ำสับปะรดเพื่อหายีสต์สายพันธุ์จำเพาะมาประยุกต์ใช้เป็นก้ำเชื้อสำหรับหมักไวน์สับปะรดที่มีคุณภาพ การทดลองเริ่มต้นด้วยการติดตามยีสต์ท้องถิ่นที่เกี่ยวข้องกับผลสับปะรดและน้ำสับปะรดคั้นสดที่ปล่อยให้เกิดการหมักแบบธรรมชาติ โดยตรวจสอบยีสต์ท้องถิ่นที่เกี่ยวข้องกับผลสับปะรดชวงอายุต่างๆ และผลสับปะรดแตกเสียหายที่เพาะปลูกในไร่สับปะรดในประเทศไทยด้วยวิธี rinsing และ enrichment จากนั้นนำโคโลนีของยีสต์ที่แยกได้มาระบุสายพันธุ์ด้วยวิธีการวิเคราะห์แบบดั้งเดิม (conventional method) ร่วมกับวิธีทางชีวโมเลกุล (molecular methods) 3 วิธี ได้แก่ PCR-RFLP ของชิ้นส่วน ITS ของ ribosomal DNA และวิธีการวิเคราะห์ sequencing บนบริเวณ 26S (D1/D2) และ ITS ของ ribosomal DNA ร่วมกับการใช้ระบบ ID 32 C จากผลการทดลองเมื่อแยกยีสต์ด้วยวิธี rinsing พบว่า จำนวนประชากรยีสต์ท้องถิ่นบนเปลือกสับปะรดเพิ่มขึ้นอย่างมีนัยสำคัญ ($p \leq 0.05$) ตามอายุของผลสับปะรดที่เพิ่มขึ้น โดยยีสต์หลักที่พบบนผลสับปะรดสมบูรณ์ของทุกชวงอายุการเก็บเกี่ยวคือ *Aureobasidium pullulans* ในขณะที่ยีสต์ที่มีคุณสมบัติในการหมักคือ *Pichia guilliermondii* ซึ่งพบเป็นยีสต์หลักบนผลสับปะรดสมบูรณ์ในระยะเก็บเกี่ยว สำหรับผลแตกเสียหาย ยีสต์ที่พบมีเพียงสายพันธุ์เดียวคือ *Zygosaccharomyces bailii* และเมื่อแยกยีสต์ด้วยวิธี enrichment พบว่ายีสต์หลักที่แยกได้จากผลสับปะรดชวงอายุคือ *P. guilliermondii* และ *Hanseniaspora uvarum* สำหรับผลแตกเสียหาย ยีสต์หลักที่พบยังคงเป็น *Z. bailii* ยีสต์สายพันธุ์อื่นที่พบเพิ่มเติมมีจำนวนไม่มากถึงแม้จะแยกด้วยการทำ enrichment culture ยีสต์เหล่านี้ได้แก่ *Candida tropicalis*, *Candida* sp., *Erythrobasidium hasegawianum* และ *Saccharomycodes ludwigii* ซึ่งแยกได้จากบางตัวอย่างในบางครั้งเท่านั้น

สำหรับการติดตามยีสต์ในการหมักน้ำสับปะรดแบบธรรมชาติ ได้ศึกษาถึงอิทธิพลของสภาพภูมิอากาศและเขตการเพาะปลูกสับปะรดต่อยีสต์ท้องถิ่นที่เกี่ยวข้องกับระบบการหมักโดยใช้ตัวอย่างผลสับปะรดจากประเทศไทยและออสเตรเลีย ตรวจสอบยีสต์ที่เกี่ยวข้องกับการหมักน้ำสับปะรดคั้นสดแบบธรรมชาติด้วยวิธีการเพาะเลี้ยงบนอาหารเลี้ยงเชื้อร่วมกับวิธีวิเคราะห์ DNA ของยีสต์ที่สกัดได้จากน้ำหมักด้วย PCR-DGGE จากผลการทดลองพบว่า *H. uvarum* และ *P. guilliermondii* เป็นยีสต์หลักที่ทำให้เกิดการหมักแบบธรรมชาติของน้ำสับปะรดจากทั้งประเทศไทยและออสเตรเลีย โดยพบ *P. guilliermondii* เป็นยีสต์หลักในช่วงเริ่มต้นของการหมัก หลังจากนั้นจำนวนยีสต์ *H. uvarum* จะเพิ่มมากขึ้นและเป็นยีสต์หลักจนกระทั่งสิ้นสุดการหมัก จำนวนประชากรของยีสต์ทั้งสองสายพันธุ์นี้อยู่ระหว่าง 5 ถึง 8 log cfu ml⁻¹ ตลอดการหมัก โดยเอทานอลที่ถูกสร้างขึ้นในระบบการหมักน้ำสับปะรดแบบธรรมชาติมีปริมาณอยู่ระหว่าง 1 ถึง 4 % (v/v)

จากนั้นนำยีสต์ที่คัดแยกได้จากการศึกษาข้างต้นที่สามารถสร้างแอลกอฮอล์ได้มากกว่า 5% (v/v) มาตรวจสอบคุณลักษณะการหมักโดยใช้น้ำสับปะรดเป็นสับเสตรท จากคุณลักษณะการหมักที่ศึกษาพบว่า *S'codes ludwigii* (SI) เป็นยีสต์ที่สามารถสร้างเอทานอลได้สูงสุดและมีลักษณะการหมักอื่นๆ ที่คล้ายคลึงกับ *Saccharomyces cerevisiae* สายพันธุ์ที่ใช้ทางการค้า และ *H. uvarum*1 (Hu1) เป็นยีสต์ที่สามารถสร้างสาร 2-phenylethyl acetate ซึ่งเป็นสารระเหยที่มีรายงานว่าให้กลิ่นหอมของดอกกุหลาบและดอกไม้ ดังนั้นจึงเลือก *S'codes ludwigii* (SI), *H. uvarum*1 (Hu1) และ *S. cerevisiae* มาประยุกต์ใช้เป็นก้ำเชื้อผสม (mixed starter cultures) สำหรับหมักไวน์สับปะรด จากผลการทดลองพบว่าก้ำเชื้อ *S'codes ludwigii* (SI) ผสมกับ *H. uvarum*1 (Hu1) มีอันตรกิริยาในทางส่งเสริมกัน โดย *S'codes ludwigii* (SI) สามารถแสดงบทบาทสำคัญในการเป็นยีสต์หลักที่สร้างเอทานอลและดำเนินการหมักจนเสร็จสมบูรณ์ นอกจากนี้ *S'codes ludwigii* (SI) ยังช่วยให้ *H. uvarum*1 (Hu1) มีชีวิตอยู่ในระบบการหมักให้นานขึ้นทำให้ยีสต์นี้มีเวลาในการสร้างระเหย 2-phenylethyl acetate ในไวน์สับปะรดได้

ภาควิชา.....เทคโนโลยีทางอาหาร.....

สาขาวิชา.....เทคโนโลยีทางอาหาร.....

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ON-ONG CHANPRASARTSUK: AUTOCHTHONOUS YEASTS
ASSOCIATED WITH PINEAPPLE WINE FERMENTATION AND
CHEMICAL CHANGES DURING FERMENTATION PROCESS. ADVISOR:
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FLEET, Ph.D., 282 pp.

The main goal of this study is to undertake investigation of the yeasts associated with spontaneous pineapple juice fermentation to find specific yeasts, which could be applied as starter cultures for quality pineapple wine fermentations. Initially, the indigenous yeasts associated with pineapple fruits and with freshly crushed pineapple juices allowing natural fermentation to occur were investigated. The indigenous yeasts associated with pineapple fruits at various stages of maturity throughout cultivation in the fields of Thailand including damaged fruits were examined for their populations and species of yeasts, performing the isolation of yeast by rinsing and enriching methods. The yeast isolates were identified by the combination of cultural and three molecular methods, PCR-RFLP analysis, sequencing analysis of the 26S (D1/D2) and ITS region of ribosomal DNA, and ID 32 C system. With rinsing method, the results showed that the population of yeast on pineapple surfaces increased in parallel with maturity development of pineapple fruits. *Aureobasidium pullulans* was found as a common yeast on intact fruits skin at every cultivation stage, whereas only fermentative yeast, *Pichia guilliermondii*, was found as the prevalent species on mature, intact fruits. On damaged fruits, only *Zygosaccharomyces bailii* was found. For the enrichment methods, *P. guilliermondii* and *Hanseniaspora uvarum* were consistently found on intact pineapple skins at every cultivation stages. On damaged fruits, *Z. bailii* was still found as the main species. Not many yeast species were additionally found on pineapple skins, even when isolated by enrichment cultures. Apart from unidentified yeast, there were just four yeast species, *Candida tropicalis*, *Candida* sp., *Erythrobasidium hasegawianum* and *Saccharomycodes ludwigii*, which were found on a few occasions.

For the investigation of the yeasts in spontaneous fermentation, pineapple samples from Thailand and Australia were studied to investigate the influence of climate and region on the yeasts associated with the fermentation systems. The yeasts associated with the spontaneous fermentations were determined by cultural isolation and PCR-DGGE analysis. Based on the data obtained from both methods, *H. uvarum* and *P. guilliermondii* were the main species similarly isolated from the fermentation systems of freshly crushed pineapple juice in all samples. *P. guilliermondii* was present as dominant species during the early stage of the fermentation, then *H. uvarum* became more prevalent until the final day of fermentation. Their populations increased from initial approximately 5 to 8 log cfu ml⁻¹ through to the end of fermentation. Ethanol generated in the system of these natural fermentations was varied between 1 to 4 % (v/v).

The yeast isolates obtained from this study were selected to investigation of their alcoholic fermentation characteristics if it generated alcohol higher than 5% (v/v). Based on the fermentation characteristics, *S'codes ludwigii* (Sl) was selected since it could produce the highest amount of ethanol content relative to the other yeast isolates. In addition, it showed the other fermentation characteristics, which were similar to a commercial *S. cerevisiae*. *H. uvarum*1 (Hu1) was also selected since it could generate 2-phenylethyl acetate, which is reported as a volatile compound giving rose and flowery odors. Therefore, these two yeast isolates and the commercial *S. cerevisiae* were applied as mixed starter cultures for pineapple wine fermentation. It was found that the mixed cultures of *S'codes ludwigii* (Sl) and *H. uvarum*1 (Hu1) had positive interaction. *S'codes ludwigii* (Sl) could play role as the main yeasts to produce the ethanol and complete the fermentation same as *S. cerevisiae*. In addition, it could extend the survival of *H. uvarum*1 (Hu1) during the fermentation allowing *H. uvarum*1 (Hu1) to carry out the fermentation and produce the 2-phenylethyl acetate in pineapple wine.

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CONTENTS

	Page
ABSTRACT (Thai).....	iv
ABSTRACT (English).....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF TABLES.....	xvi
LIST OF FIGURES.....	xix
NOMENCLATURE.....	xxiv
CHAPTER	
I INTRODUCTION.....	1
II LITERATURE REVIEW.....	3
2.1 Wine fermentation.....	3
2.1.1 Wine grape.....	5
2.1.2 Crushing and pre-fermentation treatment.....	5
2.1.3 Alcoholic fermentation.....	6
2.1.4 Malolactic fermentation.....	7
2.1.5 Post fermentation process.....	7
2.2 Fruit wine.....	8
2.3 Pineapple wine.....	10
2.3.1 Pineapple fruit.....	10
2.3.2 Pineapple wine making.....	12

2.4 Yeasts and wine fermentation.....	16
2.5 Autochthonous yeasts.....	18
2.6 Wine flavors.....	20
2.6.1 Wine flavors originated from grape and pre-fermentation.....	21
2.6.2 Wine flavors originated from fermentation.....	25
2.6.2.1 Succinic acid.....	27
2.6.2.2 Acetic acid.....	28
2.6.2.3 Fumaric acid.....	30
2.6.2.4 Malic acid.....	31
2.6.2.5 Lactic acid.....	31
2.6.2.6 Tartaric acid.....	33
2.6.2.7 Esters.....	35
2.6.2.8 Higher alcohols.....	36
2.6.2.9 Aldehydes and ketones.....	37
2.6.2.10 Lactones.....	42
2.6.2.11 Nitrogen-containing compounds.....	42
2.6.2.12 Sulfur-containing compounds.....	43
2.6.3 Wine flavors originated from aging.....	44
2.7 Autochthonous yeasts and their roles in wine flavors.....	45
2.8 Specific yeasts.....	48
2.9 Wine flavors improvement.....	50
2.10 Wine yeast determination.....	52
2.10.1 Conventional method.....	54

2.10.2 Commercial kits.....	54
2.10.3 Molecular method.....	55
2.10.3.1 Restriction Fragment Length Polymorphisms (RFLP) analysis.....	57
2.10.3.2 Sequencing method.....	58
2.10.3.3 Denaturing Gradient Gel Electrophoresis (DGGE) analysis.....	61
2.11 Wine flavor analysis.....	63
2.11.1 Non-volatile compounds.....	63
2.11.2 Volatile compounds.....	66
III ISOLATION AND IDENTIFICATION OF YEASTS ASSOCIATED WITH PINEAPPLE FRUITS AND INDIGENOUS YEASTS IN NATURAL PINEAPPLE JUICE FERMENTATION.....	70
3.1 Introduction.....	70
3.2 Materials and methods.....	71
3.2.1 Pineapple sources.....	71
3.2.1.1 Isolation of yeasts associated with pineapple fruit by rinse-plating method.....	72
3.2.1.2 Isolation of yeasts on pineapple fruit by enrichment method.....	72
3.2.2 Isolation and identification of yeasts from spontaneous fermentations.....	73

3.2.3 DNA extraction from yeasts.....	74
3.2.4 DNA amplification and primers.....	74
3.2.5 RFLP analysis.....	76
3.2.6 DGGE analysis.....	77
3.2.7 Sequencing analysis of DNA bands and yeast identification.....	78
3.2.8 Determination of fermentation profile.....	79
3.2.8.1 Determination of yeast population by cultural method.....	79
3.2.8.2 Preparation of fermented pineapple juice for HPLC analysis.....	79
3.2.8.3 Analysis of ethanol.....	79
3.2.8.4 Analysis of sugars.....	80
3.2.8.5 Analysis of organic acids.....	81
3.3 Results and discussion.....	81
3.3.1 Yeast identification.....	81
3.3.1.1 Identification of yeast isolates from Thailand by three molecular methods.....	83
3.3.1.2 Identification of yeast isolates from Australia by two molecular methods and the ID 32 C system.....	86
3.3.2 Yeasts associated with pineapple fruits	90
3.3.2.1 Yeasts isolated by rinsing method.....	90
3.3.2.2 Yeasts isolated by enrichment method.....	94
3.3.3 Yeasts in spontaneous fermentation of freshly crushed pineapple juices.....	108

3.3.3.1 Spontaneous fermentation of freshly crushed pineapple juices from pineapple in Thailand.....	109
3.3.3.2 Spontaneous fermentation of freshly crushed pineapple juices from pineapple in Australia.....	121
3.3.4 Yeast flora of fermentations.....	133
3.3.5 Chemical changed during fermentation – sugars, Ethanol production and organic acids.....	137
 IV DETERMINATION OF FERMENTATION PROFILE OF YEAST ISOLATES IN PINEAPPLE JUICE FERMENTATION.....	141
4.1 Introduction.....	141
4.2 Materials and methods.....	142
4.2.1 Yeast isolates.....	142
4.2.2 Micro-fermentation screening.....	142
4.2.3 Determination of fermentation profile.....	144
4.2.3.1 Determination of yeast population by cultural method...	145
4.2.3.2 Preparation of fermented pineapple juice for chemical analysis by HPLC	145
4.2.3.3 Analysis of ethanol.....	145
4.2.3.4 Analysis of sugars.....	146
4.2.3.5 Analysis of organic acids.....	146
4.2.4 Analysis of volatile compounds.....	147
4.2.4.1 HS-SPME extraction.....	147

4.2.4.2 GC-MS analysis.....	148
4.3 Results and discussion.....	148
4.3.1 Micro-fermentation screening.....	148
4.3.2 Fermentation profile of yeast isolates.....	150
4.3.2.1 Fermentation with <i>S. cerevisiae</i>	151
4.3.2.2 Fermentation with <i>S'codes ludwigii</i>	153
4.3.2.3 Fermentation with <i>H. uvarum</i>	155
4.3.2.4 Fermentation with <i>Z. bailii</i>	157
4.3.2.5 Fermentation with <i>Candida</i> sp.....	159
4.3.3 Concentration of organic acids during fermentation of pineapple juice by yeast isolates.....	161
4.3.3.1 Citric acid.....	161
4.3.3.2 Malic acid.....	162
4.3.3.3 Succinic acid.....	162
4.3.3.4 Lactic acid.....	163
4.3.3.5 Fumaric acid.....	163
4.3.3.6 Acetic acid.....	164
4.3.4 Analysis of volatile metabolites produced by yeasts during the fermentation of pineapple juice.....	166
4.3.4.1 Pineapple juice.....	166
4.3.4.2 Fermentation with <i>S. cerevisiae</i>	166
4.3.4.3 Fermentation with <i>S'codes ludwigii</i>	168

4.3.4.4 Fermentation with <i>H. uvarum</i>	168
4.3.4.5 Fermentation with <i>Z. bailii</i>	168
4.3.4.6 Fermentation with <i>Candida</i> sp.....	169
4.3.5 Fermentation properties of yeast isolates.....	169
4.3.5.1 Organic acids production of yeast isolates in pineapple wine fermentations.....	172
4.3.5.2 Volatile compounds production of yeast isolates in pineapple wine fermentations.....	173
V FERMENTATION OF PINEAPPLE JUICE WITH SINGLE AND MIXED STARTER CULTURES OF YEASTS.....	176
5.1 Introduction.....	176
5.2 Materials and methods.....	177
5.2.1 Determination of fermentation profile.....	179
5.2.1.1 Determination of yeast population by cultural method...	179
5.2.1.2 Preparation of pineapple wine for HPLC analysis.....	180
5.2.1.3 Analysis of ethanol, sugars and organic acids.....	180
5.2.2 Analysis of volatile compounds.....	180
5.3 Results and discussion.....	180
5.3.1 Yeast, sugar and ethanol profiles during pineapple wine fermentation by single and mixed starter cultures of yeasts.....	180
5.3.1.1 Fermentation with mixed cultures of <i>S. cerevisiae</i> and <i>H. uvarum</i>	183

5.3.1.2 Fermentation with mixed cultures of <i>S'codes ludwigii</i> and <i>H. uvarum</i>	185
5.3.1.3 Fermentation with mixed cultures of <i>S. cerevisiae</i> and <i>S'codes ludwigii</i>	187
5.3.1.4 Fermentation with mixed cultures of <i>S. cerevisiae</i> , <i>S'codes ludwigii</i> and <i>H. uvarum</i>	189
5.3.2 Organic acids of pineapple wine fermentation by mixed starter cultures.....	193
5.3.2.1 Citric acid.....	193
5.3.2.2 Malic acid.....	196
5.3.2.3 Succinic acid.....	196
5.3.2.4 Lactic acid.....	197
5.3.2.5 Fumaric acid.....	197
5.3.2.6 Acetic acid.....	198
5.3.3 Volatile compounds of pineapple wine fermented by mixed starter culture.....	199
5.3.3.1 Fermentation with mixed cultures of <i>S. cerevisiae</i> and <i>H. uvarum</i>	199
5.3.3.2 Fermentation with mixed cultures of <i>S'codes ludwigii</i> and <i>H. uvarum</i>	201
5.3.3.3 Fermentation with mixed cultures of <i>S. cerevisiae</i> and <i>S'codes ludwigii</i>	202

5.3.3.4 Fermentation with mixed cultures of <i>S. cerevisiae</i> , <i>S'codes ludwigii</i> and <i>H. uvarum</i>	203
VI CONCLUSIONS.....	205
RECOMMENDATIONS.....	210
REFERENCES.....	213
APPENDICES.....	244
APPENDIX A.....	245
APPENDIX B.....	246
APPENDIX C.....	248
APPENDIX D.....	251
APPENDIX E.....	256
VITA.....	282

LIST OF TABLES

TABLE	Page
2.1 Major compositions of grape juice.....	5
2.2 The general composition of ripe pineapple flesh.....	11
2.3 Major volatile compounds of freshly pineapple juice.....	13
2.4 The autochthonous yeasts associated with fruits and other agricultural products.....	20
2.5 The main monoterpenols in grapes.....	25
2.6 The principle higher alcohols and their amino acids precursor in wine.....	38
2.6 The principle higher alcohols and their amino acids precursor in wine (cont.).....	39
2.7 Aldehydes and ketones in wine.....	41
2.8 Some volatile compounds originated during wine aging.....	45
2.9 Multi-starter cultures used for wine fermentation.....	53
2.10 Different kits and systems used to identify foodborne yeasts.....	56
2.11 Some studies on combinations of RFLP and sequencing analysis to identify yeasts in food and beverages.....	60
2.12 Aminex® HPX-87H ion exclusion column equipped with HPLC used for chemical analysis.....	65
2.13 Types of SPME Fibers.....	68
2.14 HS-SPME coupled with GC-MS used for volatile compounds analysis.....	69

TABLE

Page

3.1	Identification of yeasts isolated from pineapple fruits and spontaneous fermentation of freshly crushed pineapple juice in Thailand.....	85
3.2	Identification of yeasts isolated from spontaneous fermentation of freshly crushed pineapple juice in Australia.....	88
3.3	Yeast isolated from pineapple skins at different cultivation stages and damaged fruits by rinse-plating method.....	91
3.4	Yeast isolated from skin of pineapple fruits at 3 months before harvesting stages by enrichment methods.....	95
3.5	Yeast isolated from skin of pineapple fruits at 2 months before harvesting stages by enrichment methods.....	96
3.6	Yeast isolated from skin of pineapple fruits at 1 months before harvesting stages by enrichment methods.....	99
3.7	Yeast isolated from skin of pineapple fruits at harvesting stages by enrichment methods.....	100
3.8	Yeast isolated from skin of damaged fruits by enrichment methods.....	101
3.9	Summary of yeast species isolated by enrichment methods from skin of pineapples at different maturity stages, including damaged fruits.....	103
3.10	Yeasts population during natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Thailand (TH1).....	111
3.11	Yeasts population during natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Thailand (TH2).....	118
3.12	Yeasts population during natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Australia (AUS1).....	123

TABLE	Page
3.13 Yeasts population during natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Australia (AUS2).....	130
4.1 Yeasts selected from the enrichment cultures and natural fermentation of pineapple sample from Thailand and their source of isolation.....	143
4.2 Fermentation characteristics of 20 yeast isolates in pineapple juice micro-fermentation.....	150
4.3 Main volatile compounds of fermented pineapple juice by the yeast isolates.....	167
5.1 The experimental designs of multi-starter cultures used in pineapple juice fermentation.....	178
5.2 Main volatile compounds of fermented pineapple juice by mixed starter cultures.....	200

LIST OF FIGURES

FIGURE	Page
2.1 Processes diagram of red and white wine production.....	4
2.2 Traditional pineapple wine fermentation processes.....	14
2.3 The main organic acids in grapes	23
2.4 The main monoterpenes and derivatives identified in grape and wine	24
2.5 Secondary compounds formation by yeast during fermentation	26
2.6 The major organic acids produced during fermentation.....	27
2.7 Formations of succinic acid.....	29
2.8 Acetic acid formation by yeast fermentation (i) and acetic acid bacteria (ii).....	30
2.9 The decomposition of malic acid by yeast fermentation.....	31
2.10 Malolactic fermentation.....	32
2.11 Decomposition of tartaric acid by lactic acid bacteria.....	34
2.12 Esterification balance of an alcohol.....	36
2.13 Biosynthesis mechanism of fatty acids.....	36
2.14 Formation of higher alcohol from amino acids (Ehrlich reactions).....	37
2.15 Formation of γ -butyrolactone.....	42
2.16 Diagram of the D1/D2 domain and ITS region of ribosomal DNA.....	58
3.1 Yeasts population during natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Thailand (TH1).....	110

FIGURE	Page
3.2 PCR-DGGE analysis of yeasts associated with natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Thailand (TH1)	112
3.3 The changes of yeasts population, % ethanol, sugars concentrations and pH during natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Thailand (TH1).....	114
3.4 Change in concentration of organic acids during natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Thailand (TH1).....	114
3.5 Yeasts population during natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Thailand (TH2).....	117
3.6 PCR-DGGE analysis of yeasts associated with natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Thailand (TH2).....	119
3.7 The changes of yeasts population, % ethanol, sugars concentrations and pH during natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Thailand (TH2).....	120
3.8 Change in concentration of organic acids during natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Thailand (TH2).....	120
3.9 Yeasts population during natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Australia (AUS1).....	122

FIGURE	Page
3.10 PCR-DGGE analysis of yeasts associated with natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Australia (AUS1)	125
3.11 The changes of yeasts population, % ethanol, sugars concentrations and pH during natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Australia (AUS1).....	126
3.12 Change in concentration of organic acids during natural fermentation of freshly crushed pineapple juice sample 1 of fruits grown in Australia (AUS1).....	126
3.13 Yeasts population during natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Australia (AUS2).....	129
3.14 PCR-DGGE analysis of yeasts associated with natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Australia (AUS2)	131
3.15 The changes of yeasts population, % ethanol, sugars concentrations and pH during natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Australia (AUS2).....	132
3.16 Change in concentration of organic acids during natural fermentation of freshly crushed pineapple juice sample 2 of fruits grown in Australia (AUS2).....	132
4.1 Yeasts population and changes in the concentrations of ethanol, sugars and pH during fermentation of pineapple juice by <i>S. cerevisiae</i>	152

FIGURE	Page
4.2	Yeasts population and changes in the concentrations of ethanol, sugars and pH during fermentation of pineapple juice by <i>S'codes ludwigii</i> (Sl).....154
4.3	Yeasts population and changes in the concentrations of ethanol, sugars and pH during fermentation of pineapple juice by <i>H. uvarum</i> 1 (Hu1).....156
4.4	Yeasts population and changes in the concentrations of ethanol, sugars and pH during fermentation of pineapple juice by <i>H. uvarum</i> 2 (Hu2).....156
4.5	Yeasts population and changes in the concentrations of ethanol, sugars and pH during fermentation of pineapple juice by <i>Z. bailii</i> (Zb).....158
4.6	Yeasts population and changes in the concentrations of ethanol, sugars and pH during fermentation of pineapple juice by <i>Candida</i> sp.1 (Cs1).....160
4.7	Yeasts population and changes in the concentrations of ethanol, sugars and pH during fermentation of pineapple juice by <i>Candida</i> sp.2 (Cs2).....160
4.8	Changes in the concentrations of organic acids during fermentation of Pineapple juice by different yeasts.....165
5.1	The changes of yeasts population, ethanol, sugar concentrations and pH of pineapple juice during fermentation with <i>S. cerevisiae</i>181
5.2	The changes of yeasts population, ethanol, sugar concentrations and pH of pineapple juice during fermentation with <i>S'codes ludwigii</i>182
5.3	The changes of yeasts population, ethanol, sugar concentrations and pH of pineapple juice during fermentation with <i>H. uvarum</i>182
5.4	The changes of yeasts population, ethanol, sugar concentrations and pH of pineapple juice during fermentation with <i>S. cerevisiae</i> and <i>H. uvarum</i>184

FIGURE	Page
5.5 The changes of yeasts population, ethanol, sugar concentrations and pH of pineapple juice during fermentation with <i>S'codes ludwigii</i> and <i>H. uvarum</i>	186
5.6 The changes of yeasts population, ethanol, sugar concentrations and pH of pineapple juice during fermentation with <i>S. cerevisiae</i> and <i>S'codes ludwigii</i>	188
5.7 The changes of yeasts population, ethanol, sugar concentrations and pH of pineapple juice during fermentation with <i>S. cerevisiae</i> , <i>S'codes ludwigii</i> and <i>H. uvarum</i>	190
5.8 Changes in the concentrations of organic acids during fermentation of Pineapple juice by multi-starter cultures	195

NOMENCLATURE

Abbreviations	Genus
<i>A.</i>	<i>Aureobasidium</i>
<i>C.</i>	<i>Candida</i>
<i>Cr.</i>	<i>Cryptococcus</i>
<i>E.</i>	<i>Erythrobasidium</i>
<i>G.</i>	<i>Geotrichum</i>
<i>H.</i>	<i>Hanseniaspora</i>
<i>I.</i>	<i>Issatchenkia</i>
<i>K.</i>	<i>Kloeckera</i>
<i>P.</i>	<i>Pichia</i>
<i>Rh.</i>	<i>Rhodosporidium</i>
<i>R.</i>	<i>Rhodotorula</i>
<i>S'codes</i>	<i>Saccharomycodes</i>
<i>T.</i>	<i>Torulaspora</i>
<i>Tr.</i>	<i>Tremella</i>
<i>Y.</i>	<i>Yarrowia</i>
<i>Z.</i>	<i>Zygosaccharomyces</i>