UNIVERSITI PUTRA MALAYSIA

FERMENTATION OF PINEAPPLE TASTE JUICE FOR THE PRODUCTION OF CITRIC ACID USING CANDIDA LIPOLYTICA ATCC 8661

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FSMB 1990 4

FERMENTATION OF PINEAPPLE WASTE JUICE FOR THE PRODUCTION OF CITRIC ACID USING *CANDIDA LIPOLYTICA* ATCC 8661

By

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Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia

September 1990

DEDICATED

TO

MY HUSBAND

GEORGE VARUGHESE

ACKNOWLEDGEMENTS

I am sincerely grateful to Universiti Pertanian Malaysia, and in particular to the Faculty of Food Science and Biotechnology for giving me this opportunity to carry out this project as a graduate programme.

I am indebted to my supervisor, Dr. Mohammad Ismail Abdul Karim for his dedicated efforts, guidance, and constant encouragement throughout the research.

I take this opportunity to thank Encik Arbakariya Ariff for his advice and suggestions in some parts of this research.

I am especially grateful to Miss Tan Hooi Kee for giving me assistance with the statistical analysis. My gratitude is also extended to Dr. Yaakob Che Man for giving me suggestions and explanation in statistics. I also thank Miss Saffiah for helping me with the computer.

Sincere gratitude is also extended to all the staff of the Faculty of Food Science and Biotechnology, especially to all in the Microbiology Section who has helped towards the success of this project.

I am also thankful to my husband, Mr. George Varughese and to my brother, Dr. Koshy Philip whose patience, understanding and encouragement had made this study successful.

Last, but not the least, I wish to thank all my family members, especially my parents and parents-in-law for being very understanding and for their constant encouragement throughout the course of this study.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	ix
LIST OF FIGURES	xiii
LIST OF PLATES	xvi
LIST OF ABBREVIATIONS	xvii
ABSTRACT	xviii
ABSTRAK	xx
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	3
Introduction	3
Citric Acid Production by Fungi	8
Fungal Strains	8
Types of Fermentation	9
Cultural Condition	12
Carbon, nitrogen and phosphate	12
Trace Elements	15
Pretreatment of Raw Materials	18
pH	21
Inoculum Development	22

Page

Aeration, Temperature and Time of Incubation 23

Additives and Stimulants 27

Citric Acid Production by Yeasts 32

Citric Acid Production by Bacteria 39

Biochemistry of Citric Acid Fermentation 41

Regulatory Aspects of Citric Acid Fermentation by A. niger 46

Regulation of Glycolysis 46

Pyruvate Metabolism 46

Tricarboxylic Acid Cycle 48

Genetic Improvement of Citric Acid Producing Microorganisms. 50

Citric Acid Production From Food and Agricultural Substrates. 53

3 MATERIALS AND METHOD 58

Raw Materials 58

Cultures 58

Inoculum 61

List of Chemicals 64

List of Equipment 65

Experiment I-A. Testing the potential capacity of C. lipolytica ATCC 8661 and A. niger NCIM 594 in producing citric acid using glucose as a standard media.. 65

Page

Experiment I-B. Effect of phosphorus on the performance of C. lipolytica and A. niger to produce citric acid (total acid) using glucose media. 66

Experiment II-A. To determine the possibility of using pineapple waste juice as a fermentation substrate for the production of citric acid. 68

Experiment II-B. Effect of methanol on the production of citric acid. 71

Experiment III. Effect of aeration on production of citric acid by C. lipolytica grown on pineapple waste in a fermentor. 72

Analysis of Fermented Material (Pulp and Pineapple Waste Juice) 75

Analysis of Raw Material (Pineapple Waste Juice) 76

4 RESULTS AND DISCUSSION 79

Approximate Analysis of Raw Pineapple Waste juice. 79

Experiment I 81

Experiment I-A. Testing the potential capability of C. lipolytica ATCC 8661 and A. niger NCIM 594 in producing citric acid (total acids) using glucose media as a standard media. 81

Experiment I-B. Effect of phosphorus on the performance of C. lipolytica ATCC 8661 and A. niger NCIM 11414 to produce citric acid (total acids) using glucose media. 85

Page

Experiment II 92

Experiment II-A. To determine of the possibility of utilising pineapple flesh juice and pineapple waste juice for the production of citric acid. 92

Experiment II-B. Effect of methanol on citric acid production from fermentation of pineapple waste juice. 98

Experiment III 110

Experiment III-A. Effect of different levels of dissolved oxygen saturation on the performance of C. lipolytica ATCC 8661 to produce citric acid from pineapple waste juice. 110

Experiment III-B. Effect of different levels of dissolved oxygen saturation on the performance of C. lipolytica ATCC 8661 to produce citric acid from pineapple waste juice added with 3% methanol. 119

SUMMARY AND CONCLUSION 128

BIBLIOGRAPHY 132

APPENDICES 144

BIOGRAPHICAL SKETCH 181

LIST OF TABLES

Table		Page
1	Uses of Citric Acid	4
2	Estimated Production of Citric Acid by Various Citric Acid Producing Countries	6
3	Effect of Different Amounts of Vegetable Oil on the Yield of Citric Acid by *A. niger* mutant 72 - 74	30
4	Influence of Adenine and Guanine Nucleolides on Citrate Accumulation	31
5	Citric Acid Producing Media	36
6	Relation Between Main Carbon Sources and Yield of Citric Acid by Various Mutant Strains of *C. lipolytica*	54
7	Analysis of the Typical Composition of Pineapple Waste Juice	80
8	Reducing Sugars of Pineapple Waste Juice (Analysis by HPLC)	80
9	Change in pH of the Glucose Base Media Fermented with *C. lipolytica* ATCC 8661 and *A. niger* NCIM 594 Over a Period of 4 Days	157
10	Change in Brix (%) of the Glucose Base Media Fermented with *C. lipolytica* ATCC 8661 and *A. niger* NCIM 594 Over a Period of 4 Days	157
11	Total Titratable Acidity (mls 0.1N NaoH)/ml Juice of the Glucose Base Media Fermented with *C. lipolytica* ATCC 8661 and *A. niger* NCIM 594 Over a Period of 4 Days	158
12	Change in pH in the Control and Media with the 2 Organisms in the Presence and Absence of 0.1% Potassium Dihydrogen Phosphate	159

Table | Page

13 Change in Brix (%) in the Control and Media with the 2 Organisms in the Presence and Absence of 0.1% Potassium Dihydrogen Phosphate 159

14 Total Titratable Acidity (mls 0.1N NaoH) in the Control and Media with the 2 Organisms in the Presence and Absence of 0.1% Potassium Dihydrogen Phosphate 160

15 Change in pH in Control and Fermented Juice with and without 3% Methanol Over a Period of 10 Days 161

16 Total Titratable Acidity (mls 0.1N NaoH/ml Juice) in Control and Fermented Juice with and without 3% Methanol Over a Period of 10 Days 162

17 Sugar Content (mg/ml) in Control and Fermented Juice with and without 3% Methanol Over a Period of 10 Days 163

18 Change in brix (%) in Control and Fermented Juice with and without 3% Methanol Over a Period of 10 Days 164

19 Citric Acid Content (mg/ml) in the Two Treatments with and without Methanol Over a Period of 10 Days 165

20 Citric Acid (mg/ml) in Fermented Pineapple Flesh Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 166

21 Citric Acid (mg/ml) in Fermented Pineapple Waste Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 166

22 Total Titratable Acidity (mls 0.1N NaoH) in Fermented Pineapple Flesh Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 167

23 Total Titratable Acidity (mls 0.1N NaoH) in Fermented Pineapple Waste Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 168

Table **Page**

24 Sugar Content (mg/ml) in Fermented and Unfermented Pineapple Waste Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 169

25 Sugar Content (mg/ml) in Unfermented and Fermented Pineapple Waste Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 170

26 Change in pH in Unfermented and Fermented Pineapple Flesh Juice Using C. lipolytica ATCC 8661 Over a Period of 4 Days 171

27 Change in pH in Unfermented and Fermented Pineapple Waste Juice Using C. lipolytica ATCC 8661 Over a Period of 4 Days 172

28 Citric Acid Content (mg/ml) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days 173

29 Total Titratable Acidity (mls 0.1N NaoH/ml Juice) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days 174

30 Change in pH in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period 10 Days 174

31 Sugar Content (mg/ml) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days 175

32 Change in Brix (%) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days 175

33 Dry Weight of Yeast (mg/ml) in Fermented Pineapple Waste Juice at 30% Dissolved Oxygen Saturation Over a Period of 10 Days 176

34 Dry Weight of Yeast Cells and Citric Acid Content at 30% Dissolved Oxygen 177

35 Citric Acid Content (mg/ml) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days Days in the Presence of 3% Methanol 178

Table | Page

36 Total Titratable Acidity (mls 0.1N NaoH/ml juice) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days in the Presence of 3% Methanol 179

37 Change in pH in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days in the Presence of 3% Methanol 179

38 Sugar Content (mg/ml) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days in the Presence of 3% Methanol 180

39 Change in Brix (%) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days in the Presence of 3% Methanol 180

LIST OF FIGURES

Figure		Page
1	The Tricarboxylic Acid Cycle	42
2	Hypothetical Pathway of Citrate Production from n-Alkanes by Yeast	47
3	Diagrammatic Representation of Fermentor	78
4	Change in pH of the Glucose Base Medias Fermented with C. lipolytica ATCC 8661 and A. niger NCIM 594 Over a Period of 10 Days	82
5	Change in Brix (%) of the Glucose Base Medias Fermented with C. lipolytica ATCC 8661 and A. niger NCIM 594 Over a Period of 10 Days	83
6	Total Titratable Acidity of the Glucose Base Medias Fermented with C. lipolytica ATCC 8661 and A. niger NCIM 594 Over a Period of 10 Days	84
7	Change in pH in the Control and Medias Fermented with the 2 Organisms in the Presence and Absence of 0.1% Potassium dihydrogen phosphate	87
8	Change in Brix (%) in the Control and Medias Fermented with the 2 Organisms in the Presence and Absence of 0.1% Potassium dihydrogen phosphate	88
9	The Total Titratable Acidity (mls 0.1N NaoH) in the Control and Medias Containing the 2 Organisms in the Presence and Absence of 0.1% Potassium dihydrogen phosphate	90
10	Citric Acid Content (mg/ml) in Fermented Pineapple Flesh Juice Inoculated with C. lipolytica ATCC 8661 Over a Period of 4 Days	93
11	Citric Acid Content (mg/ml) in Fermented Pineapple Waste Juice Inoculated with C. lipolytica ATCC 8661 Over a Period of 4 Days	94

Figure **Page**

12 Total Titratable Acidity (mls 0.1N NaoH/ml Juice) in Unfermented and Fermented Pineapple Flesh Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 96

13 Total Titratable Acidity (mls 0.1N NaoH/ml Juice) in Unfermented and Fermented Pineapple Waste Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 97

14 Sugar Content (mg/ml) in Unfermented and Fermented Pineapple Waste Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 99

15 Sugar Content (mg/ml) in Unfermented and Fermented Pineapple Flesh Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 100

16 Change in pH in Unfermented and Fermented Pineapple Flesh Juice with C. lipolytica ATCC 8661 Over a Period of 4 Days 101

17 Change in pH in Unfermented and Fermented Pineapple Waste Juice Over a Period of 4 Days 102

18 Change in pH in the Control and Fermented Juice with and without 3% Methanol Over a Period of 4 Days 103

19 Total Titratable Acidity (mls 0.1N NaoH) in the Control and Fermented Juice with and without 3% Methanol Over a Period of 10 Days 105

20 Sugar Content (mg/ml) Analysis by HPLC Using NH_2 Lichrosorb Column in the Control and Fermented Juice with and without Methanol Over a Period of 10 Days 106

21 Change in Brix (%) in the Control and Fermented Juice with and without 3% Methanol Over a Period of 10 Days 107

22 Citric Acid Content (mg/ml) in the Two Treatments (with and without Methanol) Over a Period 10 Days 108

Figure **Page**

23 Citric Acid Content in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days 111

24 Total Titratable Acidity (mls 0.1N NaoH/ml Juice) in pH in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days 113

25 The Sugar Content (mg/ml) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days 114

26 Change in Brix (%) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days 116

27 Dry Weight of Yeast at 30% Dissolved Oxygen 117

28 Dry Weight of Yeast Cells and Citric Acid Content at 30% Dissolved Oxygen 118

29 Citric Acid Content (mg/ml) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days in the Presence of 3% Methanol 120

30 Total Titratable Acidity (mls 0.1N/NaoH/ml Juice) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days in the Presence of 3% Methanol 122

31 Change in pH in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days in the Presence of 3% Methanol 124

32 The Sugar Content (mg/ml) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days in the Presence of 3% Methanol 125

33 Change in Brix (%) in Fermented Waste Juice at Different Levels of Dissolved Oxygen Saturation Over a Period of 10 Days in the Presence of 3% Methanol 126

LIST OF PLATES

Plate		Page
1	Pineapple Skin Peeling (Ripened Stage)	59
2	Bowl Cutter ADE (Type-18 West Germany)	59
3	Finely Chopped Pieces of Pineapple Skin Peelings Using a Bowl Cutter.	60
4	Coconut Screw Press	60
5	*Candida lipolytica* ATCC 8661	62
6	*Aspergillus niger* NCIM 594	62
7	*Aspergillus niger* ATCC 11414	63
8	Pineapple Waste Juice (Before Autoclaving) ..	69
9	LH Fermentor (Model 500) Capacity - 2 Liter	73

LIST OF ABBREVIATIONS

TISTR	-	Thailand Institute of Scientific and Technological Research, Bangkok, Thailand.
FTU	-	Formazine Turbidity Units.
PDA	-	Potato Dextrose Agar.
YEMA	-	Yeast Extract Maltose Agar.
rpm	-	rounds per minute.
°C	-	degrees centigrade.
psi	-	pound square inch.
mg/ml	-	milligram/milliliter.
KH_2PO_4	-	Potassium dihydrogen phosphate.
NaOH	-	Sodium hydroxide.
%	-	percentage.
HPLC	-	High Performance Liquid Chromatograph

Abstract of the Thesis Presented to the Senate of Universiti Pertanian Malaysia in Fulfilment of the Requirement for the Degree of Master of Science.

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by

JOYCE KOSHY

September 1990

Supervisor : Dr. Mohammad Ismail Abdul Karim

Faculty : Food Science and Biotechnology.

Fermentation of pineapple waste juice using *Candida lipolytica ATCC 8661* for production of citric acid was conducted. A comparison study was also carried out using the pineapple flesh juice (edible portion). Maximum production of citric acid obtained from the flesh juice and waste juice was 2.5 g/l and 3.2 g/l respectively after 4 Days under submerged fermentation using shake flasks at 30°C.

The addition of 0.1% potassium dihydrogen phosphate in the 10% pure glucose media increased the production of total acids (citric acid). Addition of 3% methanol prior to incubation into the pineapple waste juice showed a remarkable increase in citric acid production. Fermentation of pineapple waste juice using Candida lipolytica ATCC 8661 was also conducted in LH fermentor (Model 500). The effect of different levels of dissolved oxygen saturation (10%, 20%, 30% and 40%) with and without 3% methanol on citric acid production of pineapple waste juice was studied over a 10-day fermentation period at 30°C. The maximum citric acid production was 8.2 g/l in sample with 3% methanol having 40% dissolved oxygen saturation after 8 days of fermentation. Maximum of biomass production of 12.37 mg/ml juice (dry cell weight) was obtained from fermented pineapple waste juice inoculated with C. lipolytica ATCC 8661 having 30% dissolved oxygen saturation after 8-10 days of fermentation.

Abstrak thesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia memenuhi syarat untuk memperolehi Ijazah Master Sains.

PENGHASILAN ASID SITRIK DARIPADA KULIT NENAS DENGAN MENGGUNAKAN *CANDIDA LIPOLYTICA ATCC 8661*

Oleh

JOYCE KOSHY

September 1990

Penyelia : Dr. Mohammad Ismail Abdul Karim

Fakulti : Sains Makanan dan Bioteknologi

Kajian fermentasi ke atas jus kulit nenas dengan menggunakan *Candida lipolytica ATCC 8661* bagi penghasilan asid sitrik telah dijalankan. Perbandingan juga telah dijalankan dengan menggunakan jus isi nenas (bahagian yang boleh dimakan). Penghasilan asid sitrik yang maksimum dari jus isi dan jus kulit ialah 2.6 g/l dan 3.2 g/l masing-masing selepas 4 hari fermentasi rendaman dijalankan pada suhu 30°C.

Penambahan 0.1% kalium dihydrogen phosphate dalam jus kulit nenas meningkatkan penghasilan asid sitrik. Penambahan 3% metanol pada jus kulit sebelum inkubasi menunjukkan peningkatan yang jelas dalam penghasilan asid sitrik.

Fermentasi jus kulit nenas menggunakan Candida lipolytica ATCC 8661 telah dijalankan di dalam 'LH Fermentor'. Kesan paras kepekatan oksigen terlarut yang berbeza (10%, 20%, 30%, 40%) dengan penambahan atau tidak 3% metanol telah dikaji selama 10 hari jangka masa fermentasi pada suhu 30°C. Penghasilan asid sitrik yang maksimum iaitu 8.2 g/l dalam sampel yang mempunyai 3% metanol pada konsentrasi 40% oksigen terlarut selepas 8 hari fermentasi. Penghasilan biomass yang tertinggi 12.37 mg/ml jus (berat kering yis) diperolehi dari fermentasi jus kulit nenas yang diinokulasikan dengan C. lipolytica ATCC 8661 yang mempunyai konsentrasi oksigen terlarut 30% selepas 10 hari fermentasi.

CHAPTER 1

INTRODUCTION

Citric acid ($CH_2COOH\ COH\ COOH\ CH_2COOH$) a tricarboxylic acid was first isolated from lemon juice and crystallized in 1784 by Scheele. It is found as a natural constituent of a variety of fruits. However, members of the citrus family are especially rich in this organic acid. Citric acid extracted from fruits is commercially known as natural citric acid in contrast to the citric acid produced by microbial fermentation. Until the early days of this century, citric acid was produced from lemon juice although Wehmer (1893) had described this organic acid as a metabolic product of moulds of the genera Penicillium and Mucor. Today, most of the citric acid used in food and other industries comes from fungal fermentations. Although chemical synthesis of this organic acid is possible, no competitive synthetic process that is superior to fungal fermentations has been developed (Kapoor, et al, 1982).

Malaysia has been relying on the import of citric and other organic acids for its use in the beverage, food, industrial and pharmaceutical industry. The importation of citric acid in 1977 was M$3,603,196 (Malaysian ringgit), and has increased to M$ 7,955,570 in 1984 (Anonymous, 1977;

Anonymous, 1984). The exploitation of fermenting locally available raw materials to produce these acids especially with emphasis on citric acid production may save the country in foreign exchange earnings. Study on the utilisation of agricultural wastes especially on the usage of pineapple waste juice for fermentation of citric acid is being looked into in this study. The use of selected strains of microorganisms with various fermentation parameters for production of citric acid is also being studied.

The objectives of this research are:

1. To study the utilisation of pineapple skin peeling waste juice as a fermentation substrate for the production of citric acid using Candida lipolytica ATCC 8661, Aspergillus niger NCIM 594 and Aspergillus niger ATCC 11414.

2. To screen the potential citric acid producing strains.

3. To study the effect of potassium dihydrogen phosphate and methanol on citric acid production.

4. To study the effect of different levels of dissolved oxygen concentration with and without the addition of methanol (as an additive) on citric acid production using a fermentor.

CHAPTER 2

LITERATURE REVIEW

Introduction

Citric acid is produced fermentatively by many moulds and yeasts of which Aspergillus niger has been widely used. However, besides A. niger, many other species have been considered as citric acid producers, and many trials have been done to increase the potentialities of strains of A. niger, for instance, by exposure of the spores to ultra-violet irradiation and x-rays. Citric acid is used in the preparation of many industrial products and the commercial use of citric acid have led to an increasing demand for it.

Uses

Citric acid has a variety of uses. About 70% of the citric acid produced is used in the food and beverage industry, about 12% in pharmaceuticals, and about 18% in other industrial applications (Kapoor et al., 1982). The food and beverage industry uses citric acid mostly as an acidulant because of its high solubility, extremely low toxicity and imparts a pleasant sour taste. The major end uses of citric acid or its esters and salts is shown in Table 1.