

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Massey University Library  
Thesis Copyright Form

Title of thesis: *Microbiological solid state fermentation of apple pomace by yeast and fungi*

- (1) (a)  I give permission for my thesis to be made available to readers in Massey University Library under conditions determined by the Librarian.
- (b) I do not wish my thesis to be made available to readers without my written consent for ... months.
- (2) (a)  I agree that my thesis, or a copy, may be sent to another institution under conditions determined by the Librarian.
- (b) I do not wish my thesis, or a copy, to be sent to another institution without my written consent for ... months.
- (3) (a)  I agree that my thesis may be copied for Library use.
- (b) I do not wish my thesis to be copied for Library use for ... months.

Signed

*H. Rahmat*

Date

*28/3/91*

The copyright of this thesis belongs to the author. Readers must sign their name in the space below to show that they recognise this. They are asked to add their permanent address.

NAME AND ADDRESS

*H. RAHMAT  
89-C, Cook St,  
Rosedale North*

DATE

*28/3/91.*

MICROBIOLOGICAL SOLID STATE  
FERMENTATION OF APPLE POMACE  
BY YEAST AND FUNGUS

A thesis presented in partial fulfilment of the requirements for the degree of Master of Technology in Biotechnology at Massey University, Palmerston North, New Zealand.

by HISHAMUDIN RAHMAT  
1991

## Abstract

The enrichment of apple pomace prepared by the mechanical juice extraction process using *Candida utilis* Y15 was demonstrated. The organic protein content increased from 4.80% to 6.51% per dry weight of apple pomace with near total exhaustion of reducing sugar available. Using regression equation based on crude fibre content of the fermented apple pomace, the pomace may be more suitable as a feed for ruminants than for monogastrics animals.

The citric acid production by *Aspergillus niger* NRRL 328 on apple pomace prepared by the enzymatic juice extraction process was also demonstrated. *Aspergillus niger* NRRL 328 was found to produce limited amounts of citric acid. Additions of three percent methanol (v/w) stimulated citric acid production significantly. Highest level of citric acid production was observed with addition of 4% (v/w) oil and 3% methanol, which was approximately 44% of the sugar consumed. The production of citric acid seemed to be nitrogen limiting. No citric acid was produced with any exogenous nitrogen addition. The optimum inoculum size was found to be  $1 \times 10^5$  to  $1 \times 10^6$  spores per 20 gram of apple pomace.

## Acknowledgement

I would like to thank the following people :-

- Dr. Pak-Lam Yu - for his meticulous and dedicated supervision

- Dr. G. Manderson - for his help and advice in the preparation of this thesis

- Dr. Ian Maddox - for his encouragement and also for being more than an administrator

- Dr. William Smith (Monogastrics Centre) - for his advice and help

- Dr. Margaret Wilson (Biochemistry Dept.) - for being so supportive, helpful and accommodating - thanks for the book.

- Jon Marks and Mark Curphey (New Zealand Apple & Pear Marketing Board) - for the technical support and advice.

- Anne-Marie, Janice, John, John, Wayne & Bruce - What can I say; except I didn't bust the HPLC machine on purpose. Anyway, thanks heaps!

- Dave & Rosemary (Animal Science Lab.) for showing the ins- and -outs of feed testing

- All Biotech. postgraduates and honorary postgraduates  
- Too many to mention. You know who you are. Thanks guys for making my life at Massey bearable and almost fun. Cryn asked me to mention her name - CRYN

- My flatmates - Stephen, Mary, Garry & Matthew - for

putting up with my constantly changing mood

- MUM & DAD and the whole clan in Malaysia, also not forgetting my little sis somewhere in Valparaiso, Indiana, America

- Yatie - for her encouragement (forcefully at times!) and her delicious and scrumptious Spaghetti Goreng. Thanks!

HISHAM RAHMAT 31st. March 1991.

- 'The beginning of a new chapter in life' -

## Table of Contents

	page
Abstract	i
Acknowledgements	ii
Table of Contents	iv
List of Figures	x
List of Tables	xi
1 Introduction	1
1.1 Landfill	3
1.2 Stockfeed	3
1.3 Microbiological modification of stockfeed	4
1.4 Pectin production	4
1.5 Organic acids and fuel production	5
1.6 Objective of thesis	5
2 Literature Review	7
2.1 Protein enrichment of apple pomace	7
2.1.1 World outlook for stockfeed	7
2.1.2 Apple pomace as stockfeed	10
2.1.3 Single cell protein	11
2.1.4 Microorganisms	13

2.1.4.1 Bacteria	14
2.1.4.2 Yeasts	15
2.1.4.3 Fungi	15
2.1.4.4 Algae	16
2.1.5 Carbon sources for SCP production	17
2.1.5.1 Renewable resources	19
2.1.5.1.1 Saccharide substrates	20
2.1.5.1.2 Polysaccharide substrates	20
2.1.5.1.2.1 Starch	20
2.1.5.1.2.2 Lignocellulosic raw material	22
2.1.6 Energy sources	24
2.1.7 Sources of nitrogen	25
2.1.8 Sources of minor elements	25
2.1.9 Vitamins	26
2.1.10 Fermented apple pomace as stockfeed	27
2.1.11 Effect of heating on feed	28
2.1.12 Feeding of heat damaged material to animals	30
2.1.13 Data on feed	31
2.2 The production of citric acid by <i>Aspergillus</i> <i>niger</i>	33
2.2.1 Mechanism of citric acid accumulation in microorganisms	36
2.2.2 Environmental factors	38
2.2.2.1 Organisms	38
2.2.2.2 pH	40
2.2.2.3 Temperature	41
2.2.2.4 Nutrients	42



2.2.2.5 Trace metals	45
2.2.2.6 Methanol	45
2.2.3 Commercial production of citric acid	46
2.2.3.1 Submerged process	46
2.2.3.2 Surface process	47
2.2.3.3 Koji process	49
3 Materials and Methods	51
3.1 Materials	51
3.1.1 Microbiological media	51
3.1.2 Chemicals	51
3.1.3 Organisms	51
3.1.4 Apple pomace	52
3.1.5 Fermentation containers	53
3.1.5.1 Fermentation by <i>Candida utilis</i>	53
3.1.5.2 Fermentation by <i>Aspergillus niger</i>	53
3.2 Methods	53
3.2.1 Media preparation	53
3.2.2 Media, chemicals and apparatus	
sterilisation	53
3.2.3 Cleaning of glassware	54
3.2.4 Cell cultivation	54
3.2.5 Cell harvesting	54
3.2.6 Fermentation	55
3.2.6.1 Fermentation by <i>Candida utilis</i>	55
3.2.6.1.1 pH measurement	58
3.2.6.1.2 Cell count	58
3.2.6.1.3 Total nitrogen determination	59

3.2.6.1.3.1	Kjeldahl digestion procedure	59
3.2.6.1.3.2	Determination of the nitrogen content of digest	60
3.2.6.1.4	Extraction of soluble sugar and inorganic nitrogen	63
3.2.6.1.5	Total reducing sugar analysis	63
3.2.6.1.6	Inorganic nitrogen analysis	65
3.2.6.1.7	Organic nitrogen analysis	65
3.2.6.2	Fermentation of <i>Aspergillus niger</i>	65
3.2.6.2.1	Methanol additions	66
3.2.6.2.2	Ammonium sulphate additions	66
3.2.6.2.3	Natural oil additions	66
3.2.6.2.4	Inoculum size	67
3.2.6.2.5	Temperature of fermentation	67
3.2.6.2.6	Moisture content of apple pomace	67
3.2.6.2.7	Extraction of citric acid and sugar	68
3.2.6.2.8	Citric acid determination	68
3.2.7	Drying of apple pomace	68
3.2.8	Grinding of apple pomace	69
3.2.9	Freeze-drying of apple pomace	69
3.3.0	Determination of FDNB-available lysine	70
3.3.1	Stockfeed analysis	74
3.3.1.1	Ash and organic matter	74
3.3.1.2	Gross energy	75
3.3.1.3	ADF, NDF and lignin determination	75

3.3.1.4 Fat by Soxhlet method	79
3.3.1.5 Amino acid analysis	80
3.3.1.6 Microbiological analysis	80
3.3.1.6.1 Total aerobic count	81
3.3.1.6.2 Fungal spores	81
3.3.1.6.3 Enterobacteriaceae	81
3.3.1.6.4 Clostridia group	81
3.3.1.6.5 Enterococci	82
4 Fermentation of apple pomace with <i>Candida utilis</i>	83
4.1 Biochemical analyses of wet fermented apple pomace	83
4.2 Effect of drying on apple pomace	90
4.3 Stockfeed analyses of dried fermented apple pomace	92
5 Citric acid fermentation from apple pomace by <i>Aspergillus niger</i>	100
5.1 Effect of methanol additions	100
5.2 Effect of ammonium sulphate additions	101
5.3 Effect of spore inoculum	102
5.4 Effect of natural oil additions	102
5.5 Effect of temperature of fermentation	103
5.6 Effect of moisture content of apple pomace	104
6 Conclusion	111
Bibliography	113



## List of figures

Figure		page
1	A process flow diagram (Clear apple juice concentrate)	2
2	A flow digram of apple peel powder production.	6
3	Metabolic pathways of citric acid cycle.	37
4	Fermenter (frontal view).	56
5	Fermenter (side view).	57
6	Plot of relative pH and $\log_{10}$ cfu yeast / gram wet pomace vs time.	86
7	Plot of sugar conc. and $\log_{10}$ cfu yeast vs time.	87
8	Plot of nitrogen conc. and protein conc. vs time.	88
9	Plot of available lysine vs drying temperature.	91

## List of tables

Table		page
1	World's available protein (gram) per capita per day.	8
2	World meat consumption.	10
3	Fermentation characteristics of apple pomace	85
4	Nutritive evaluation of fermented apple pomace.	93
5	Amino acid profile of fermented apple pomace.	94
6	Composition of various selected feed.	98
7	Microbiological analysis of fermented apple pomace.	99
8	Effect of methanol addition.	105
9	Effect of ammonium sulphate addition.	106
10	Effect of spore inoculum.	107
11	Effect of natural oil addition.	108
12	Effect of temperature of fermentation.	109
13	Effect of moisture content of apple pomace.	110
A1	Analysis of available lysine content of apple pomace at various drying temperature.	A1

# 1 Introduction

The New Zealand Apple and Pear Marketing Board currently processes approximately 150,000 tonnes of apple pomace per year. Of this, a waste stream of 35,000 tonnes a year is generated from the juice extraction operation. This residue, apple pomace, consists of flesh, skins, stalks and pips has a high sugar level, about eighty percent moisture content and poses a considerable waste disposal problem. At present, it is trucked from factories to landfill and some is used as pigfood.

A process flow diagram (Clear apple juice concentrate production) is shown in Figure 1. In 1992 apple season, the Board will change its juice extraction operation from a mechanical to an enzymic process whereby the pomace will undergo enzymic degradation and water extraction to liberate sugars. The modified process will also include screening of the pomace so that separate fractions are produced i.e. dejuiced flesh and a mixed of pips, stalks and peel fraction. Enzymes used will be predominantly pectolytic but some side-activities including hemicellulases, arabinases and cellulases are present. The resulting pomace has significantly less sugar and potentially a modified polysaccharides composition (Marks, J., personal communication).

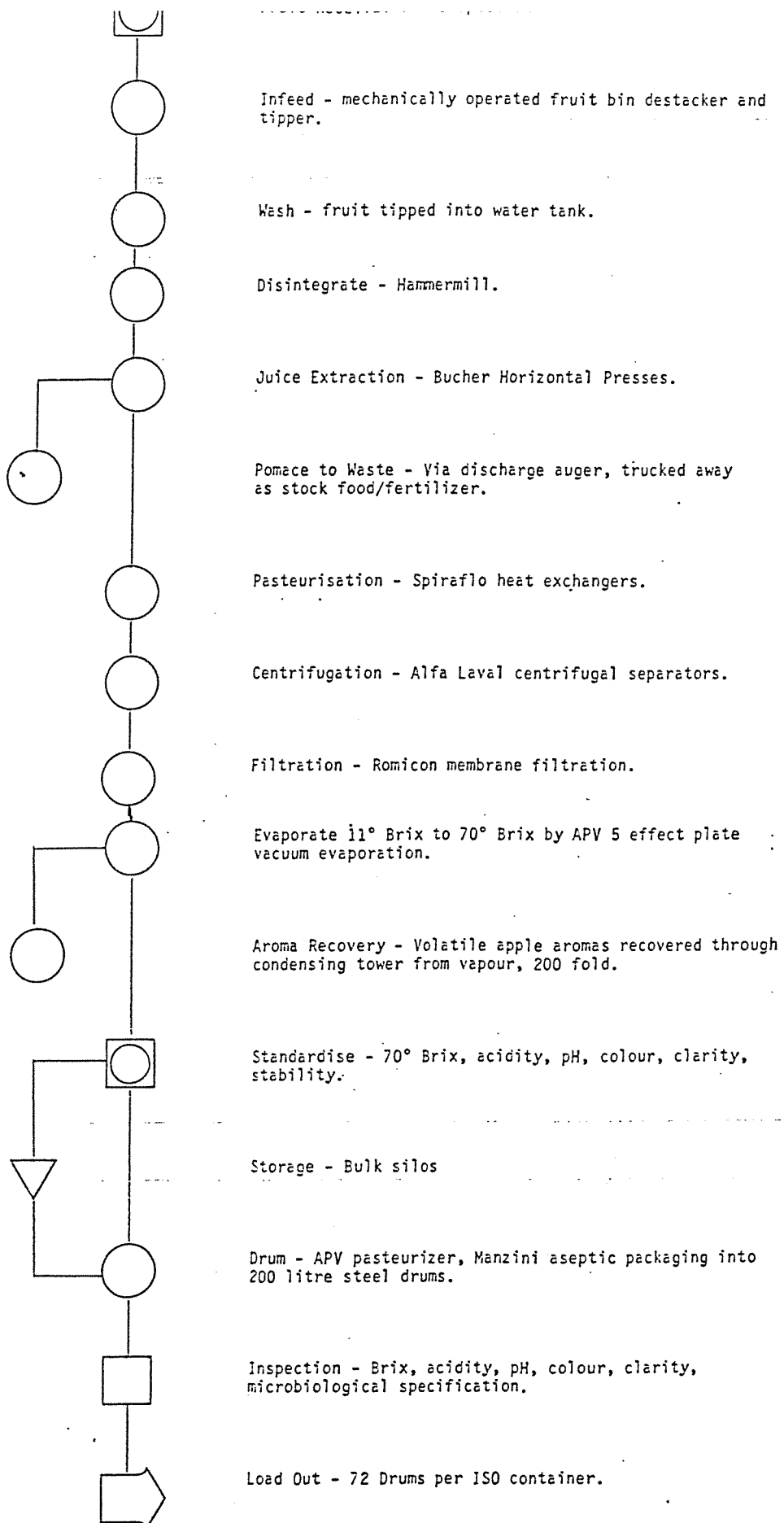


Figure 1 A process flow diagram (Clear apple juice concentrate) - (New Zealand Apple and Pear Marketing Board).



(N.B. From this point onwards, apple pomace from mechanical and enzymic juice extraction operation will be termed old and new apple pomace respectively).

Disposing apple pomace economically has always been a problem. Some of the possibilities of disposing apple pomace are as follows:-

- i) Landfill
- ii) Stockfeed
- iii) Microbiological modification for stockfeed
- iv) Pectin production
- v) Organic acids and fuel production

### **1.1 Landfill**

This method of disposal is currently being employed by the New Zealand Apple and Pear Marketing Board. Alternative options are currently being investigated by the board since this method poses ecological and environmental problem.

### **1.2 Stockfeed**

Apple pomace itself is not a very nutritious stockfeed due to lack of certain important nutrients notably protein, assimilable carbohydrate and vitamins and also seasonal availability. It also faces competition from existing stockfeed in terms of pricing. Due to its high moisture content of about eighty percent, the cost of drying could be very high and this limits its potential as a cheap stockfeed.

### 1.3 Microbiological modification for stockfeed

Numerous investigators have investigated the potential for microbiologically modifying or enriching pomace as stockfeed. Rossi *et al.* (1988) has shown that a selected strain of the fungus *Fusarium culmorum* can be grown on a mixture of orange peel powder and wheat straw. The fungal protein was up to 16.32 gram/kg pomace after sixty hour of propagation with an average productivity of 0.16 gram/kg pomace/hour. Solomon *et al.* (1988) has shown that the growth of *Saccharomyces cerevisiae* could be supported on cashew apple pomace with the production of about forty percent protein. Examples of other investigations include the use of sugar beet pulp (Grajek, 1988; Bajon *et al.*, 1985), wheat straw (Laukevics *et al.*, 1984), apple pomace (Hang *et al.*, 1988b), palm oil solid fraction (Martinet *et al.*, 1982), rice straw waste (Han *et al.*, 1974; Han *et al.*, 1976; Han *et al.*, 1978), olive black water (Ercoli *et al.*, 1983), pineapple cannery effluent (Prior, 1984), shellfish waste chitin (Revah-Moiseev *et al.*, 1981) and cheese whey (Sandhu *et al.*, 1983).

This option of utilizing apple pomace is a promising one since it may produce a higher quality but cheap protein source suitable as a substitute for existing animal feeds.

### 1.4 Pectin production

Pectin is a mixture of methyl esterified galacturonan, galactan and araban. The galacturonan molecules are linked chemically to some of the galactan and araban molecules.

Pectin is mainly used as a gelling agent in the preparation of jellies and similar food products (Merck, 1968).

Bomben *et al.*(1971) and Bomben *et al.*(1973) have described pectin production from apple pomace. The high pectin content of apple peel made itself potentially suitable for use as thickening and flavouring agent in apple pies to replace starch that is presently used as a thickener. A flow diagram of process for making apple peel powder is shown in Figure 2. Since apple peel is only about fifteen percent of total apple pomace, this process is incomplete and could still pose an environmental problem.

### **1.5 Organic acids and fuel production**

Hang (1982) and Jewell *et al.*(1984) reviewed the potential uses of apple pomace for production of fuels and food-grade chemicals. These include ethanol, biogas (primarily methane gas) and citric acid. Production of ethanol and biogas would not be commercially viable since it will face competition from a cheaper source of energy from petro-chemical industry. The production of citric acid could be a viable option since it is a widely used acid and currently New Zealand is importing all the citric acid need for the food industries.

### **1.6 Objective of this thesis**

Based on the above informations, microbial protein for stockfeed and citric acid production on apple pomace will be investigated further in this study.

FLOW DIAGRAM OF PROCESS FOR MAKING APPLE PEEL POWDER

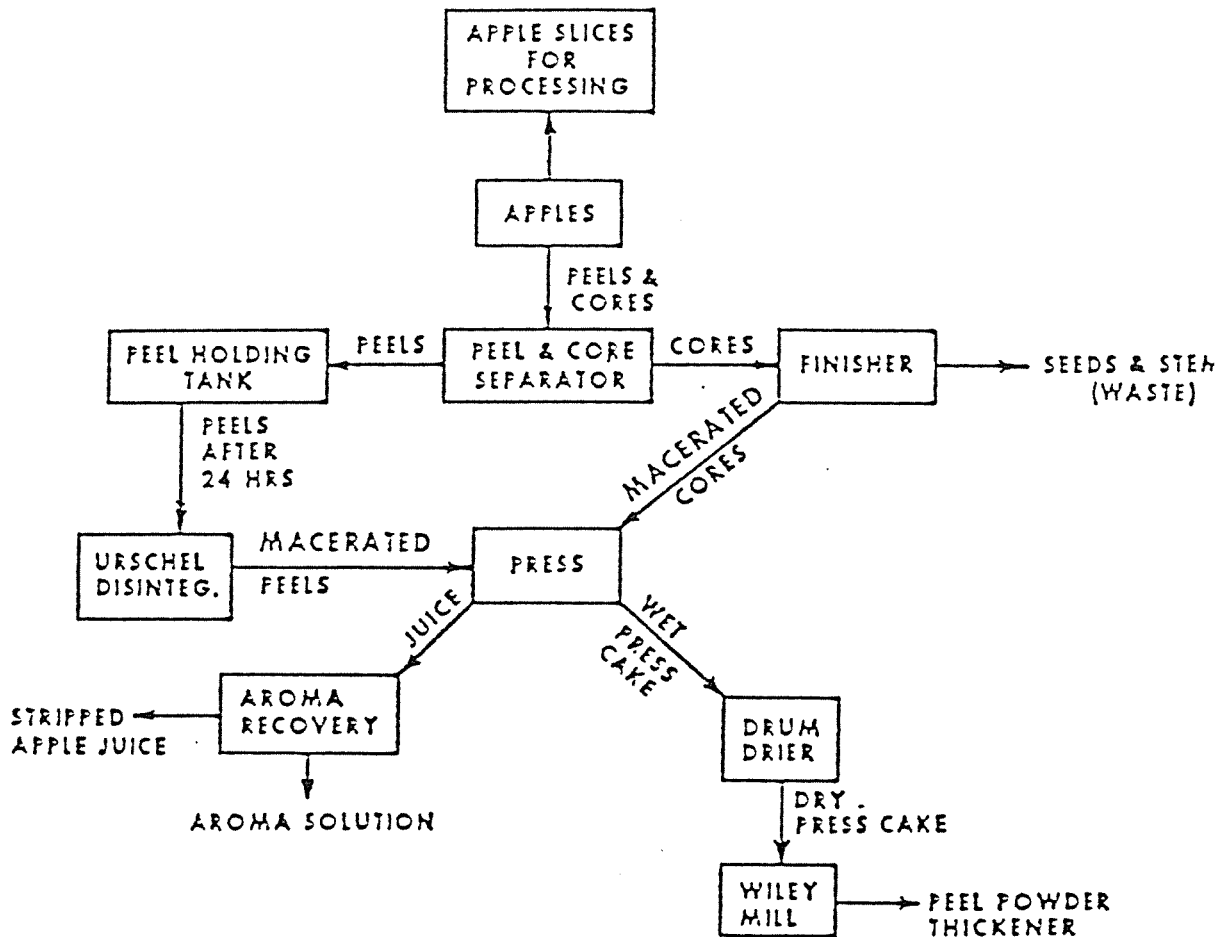


Figure 2 A flow diagram of apple peel powder production (Bomben et al., 1973).