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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 1×10^5 to 1×10^6 spores per 20 gram of apple pomace.

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HISHAM RAHMAT 31st. March 1991.

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

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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).

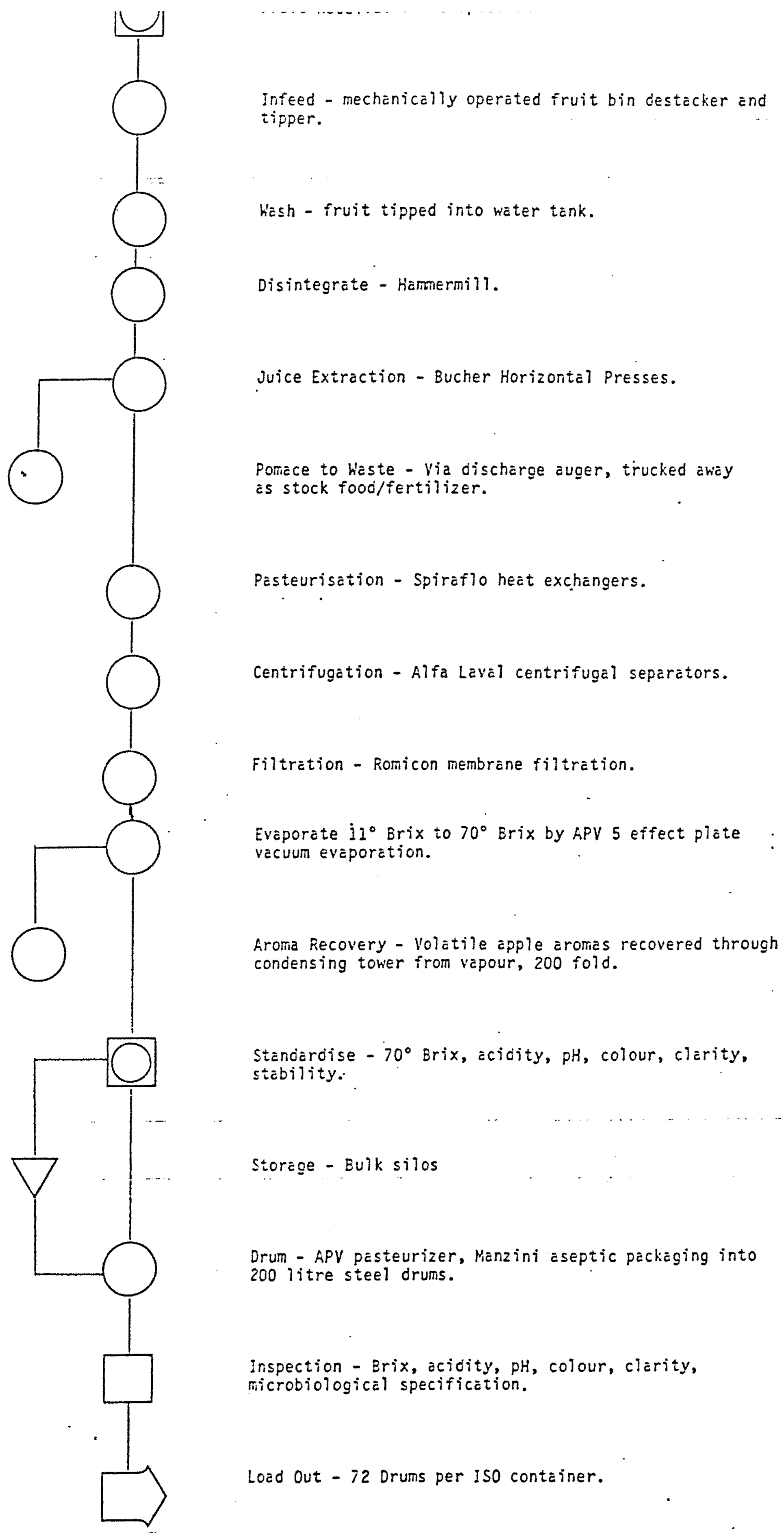

Figure 1 A process flow diagram (Clear apple juice concentrate) - (New Zealand Apple and Pear Marketing Board).

(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.

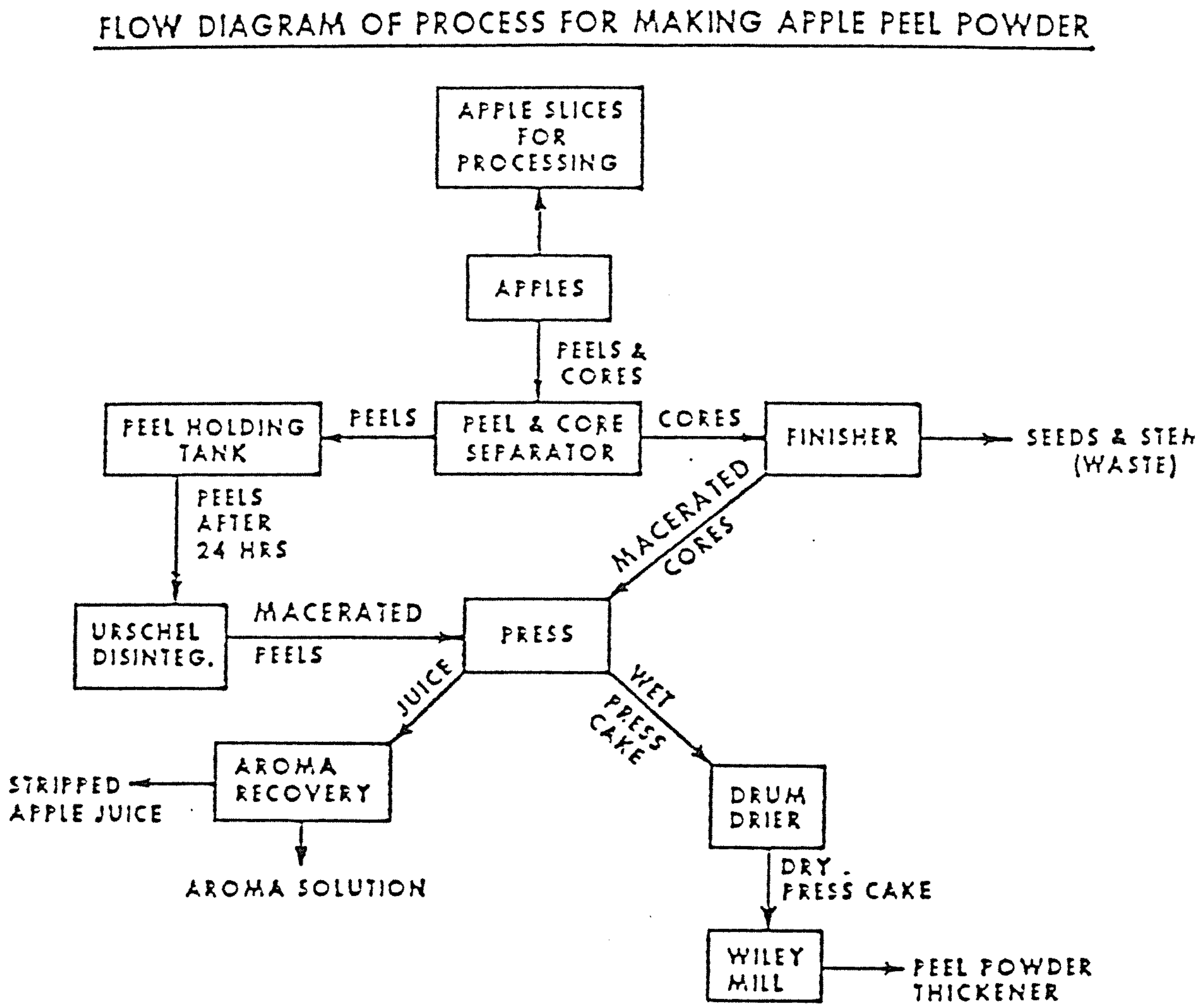

Figure 2 A flow diagram of apple peel powder production (Bomben *et al.*,1973).