THE SOLID STATE FERMENTATION OF APPLE POMACE USING YEASTS TO PRODUCE AN IMPROVED STOCK FEED SUPPLEMENT

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Abstract

Apple pomace is a waste stream generated from the apple juice extraction process and constitutes about 25% of the original fruit (Walter and Sherman, 1976). It contains a large amount of water and sugars, has a low pH and a small amount of protein. The total production of apple pomace in New Zealand is 2.7×10^4 tonnes/year. At present, it is mainly disposed of by transportation to landfill areas, with a minor portion being used locally as a pig feed supplement. However, this main disposal method is a major cost and is also of considerable environmental concern. As the quantity of pomace produced is forecasted to increase gradually over the next five years, alternative treatments and disposal options will become necessary.

This project involved the solid state fermentation of apple pomace with the aim of producing an improved stock feed supplement. The fermentations were conducted using a variety of yeasts with the purpose of improving the nutritional value by increasing the crude protein content. The effects of unsterilised media, moisture content and nitrogen addition were also addressed.

Sterilisation of the apple pomace medium prior to yeast inoculation was found to be necessary due to the superior growth characteristics of a yeast from the natural biota. This yeast was isolated and identified as *Kloeckera apiculata*.

The growth of *Kloeckera apiculata* on sterilised apple pomace was superior to that exhibited by *Candida utilis* Y15, *Saccharomyces cerevisiae* Y10 and *Yarrowia lipolytica* IFO1659. *Schizosaccharomyces pombe* H115 grew poorly on the apple pomace medium.

A reduction in the moisture content of the apple pomace medium from 80% to 65% was found to have little effect upon the growth characteristics of *C. utilis*, *Kl. apiculata* and *Sacch. cerevisiae*.

Ammonium hydroxide was the most effective nitrogeous growth substrate at improving the growth of *Kl. apiculata*, when used as a medium supplement. The growth of *C. utilis* benefited most from the addition of ammonium sulphate.

Kl. apiculata growth on apple pomace supplemented with 1% v/w 2 N ammonium hydroxide achieved a maximum crude protein content of 3.5%, measured on a dry weight basis, after 48 hours. *Kl. apiculata* growth on pomace supplemented with 1% v/w 7.8 N ammonium hydroxide achieved a maximum protein content of 7.2%, measured on a dry weight basis, after 72 hours.

Comparison of the amino acid profile of the microbially modified apple pomace (7.2% protein) with amino acid profiles recommended for growing pigs and breeding pigs revealed a deficiency in nearly all amino acids.

This research indicates that the increased protein content of the apple pomace, due to yeast propagation,

is still insufficient to qualify it as a suitable stock feed supplement. However, research into the effects of other fermentation parameters may lead to further improvements in yeast growth.

As the pig industry is potentially the greatest market in New Zealand for a microbial biomass product such as this, feeding trials with growing and breeding pigs are essential to determine its nutritional value. These trials would have a major bearing on determining the commercial prospects of this apple pomace feed product.

However, before any further research is conducted, consideration must be given to a new process which has been proposed for the extraction of apple juice. This process would result in an altered apple pomace waste stream and, if it was adopted for future commercial use, may reduce the applicability in New Zealand of the research results described in this thesis.

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TABLE OF CONTENTS

PAGE

2 MATERIALS AND METHODS

LIST OF FIGURES

FIGURE PAGE

LIST OF APPENDIX FIGURES

LIST OF TABLES

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Apple pomace is the residue left after juice extraction and represents about 25% of the original fruit (Walter and Sherman, 1976). It contains a large amount of water and sugars, has a low pH and a small amount of protein.

The New Zealand Apple and Pear Marketing Board processes approximately 1.5×10^5 tonnes of apples a year. From this apple pomace waste streams of 1.1×10^4 and 1.6×10^4 tonnes/year are generated from the juice extraction operations in Nelson and Hastings, respectively. This quantity is forecasted to increase gradually over the next five years to 3.5×10^4 tonnes/year (J. Marks, personal communication).

At present the pomace is disposed of by trucking to landfill sites with a small amount being used as pig food. However, disposal in landfill areas is costly and poses a serious environmental problem considering the volume produced and the high chemical oxygen demand, which ranges between 250 and 300 g/kg (Hours *et al.*, 1985). With increasing quantities being produced, alternative treatments and disposal options will become necessary.

A number of options have been studied but no literature has been found concerning their actual use in the apple processing industry.

The use of apple pomace as an animal feed has generally been regarded as unfeasible due to its low protein content (Hours *et al.*, 1985) and digestive troubles produced in some animals (Fontenot *et al.*, 1977; Rumsey, 1979). However, the chemical composition of pomace (Table 2) suggests that with the large concentrations of soluble and insoluble sugars available, protein enrichment by microbial growth would be possible.

The work undertaken in this thesis was aimed at improving the feed value of apple pomace by increasing its protein content. The protein enrichment was achieved by solid state fermentation of the pomace with a variety of yeasts. The effects of unsterilised media , moisture content and nitrogen addition were also monitored.

1.2 COMPOSITION OF APPLE POMACE

Apple pomace at a moisture level of approximately 80% is a solid, having no free water. Apple pomace composition (Table 2) will vary with apple varieties, growth conditions, harvesting, storage and juice extraction processes.

Table 2. Composition of Apple Pomace

	% Dry Weight	% Wet Weight
Moisture Content:	-	78-82
Carbohydrate:		
Total	72	14.4
Soluble		
(glucose and fructose)	46.2	9.2
Insoluble		
(hexose)	13.2	2.6
(pentose)	15.2	3.0
Protein (N × 6.25):	5.0	1.0
Fat:	4.0	0.8
Pectins:	8.0	1.6
Ash:	1.0	0.2
pH:	3.5	
Minerals:		
N	0.870	0.174
K	0.650	
P	0.105	
Ca	0.082	

(Table 2. cont.....)

	% Dry Weight	% Wet Weight
Mg	0.056	
Cl	0.060	
S	0.047	
Na	0.005	
Fe	0.004	
Al	0.001	
Mn	0.001	
Zn	0.001	
Cu	0.001	

(Figures supplied by the New Zealand Apple and Pear Marketing Board, Hastings, New Zealand, 1988).

1.3 PROCESSING AND DISPOSAL OPTIONS

As has already been mentioned, the major disposal method of apple pomace in New Zealand has been the transportation to landfill sites. This is also true for pomace produced in foreign countries (Etheridge and Jewell, 1988; Hours *et al.*, 1985). However, this disposal option is of major environmental concern due to the increasing quantities of pomace being produced and its high COD. As other disposal methods are also limited, the processing of apple pomace to produce a marketable and profitable product is most desirable. A summary of some processing options follows, with the processes divided into non-fermentative and

fermentative catagories. Although many of these operations still produce waste streams of utilised pomace these effluents will have a reduced COD and often a large reduction in bulk.

1.4 NON-FERMENTATIVE PROCESSES

1.4.1 Apple Press Cake Powder

As a food ingredient, apple press cake powder (apple pomace minus the pips and stalks) may have some useful attributes. The peel contains considerable apple aroma (Guadagni *et al.*, 1971) and the high pectin content (approximately 1.6% on a wet weight basis) makes the press cake potentially suitable as a thickening and texturising agent.

Bomben *et al.* (1971) described a process in which apple press cake is converted into a thickening and flavouring agent suitable for use in apple pies in place of starch. This process involved drying the press cake then grinding it into a fine powder. Drum-drying and air-drying showed poor aroma retention and, as a result, the flavour of pies made with the press cake powder often required the addition of apple aroma concentrate. In addition, the press cake powder gave the pie filling a brownish colour and, with some apple varieties, a grainy texture in contrast to the smooth, glossy appearance of the starch-thickened fillings. Bomben and Guadagni (1973) suggested that the problems associated with the grainy texture may be alleviated

by grinding the press cake finer. With the necessary use of apple aroma concentrate in conjunction with the apple press cake powder this is unlikely to be feasible product.

Factors influencing the development of aroma in apple peels were found to be harvest maturity, variety, cold storage history, room temperature ripening and storage temperature (Guadagni *et al.*, 1971).

1.4.2 Extraction of Edible Fibres

The composition of apple pomace and the known hydrocolloidal ability of some of its components, such as pectins and pentosans, make it a likely source of general purpose hydrocolloids for use in low-fibre, fabricated foods. A study of the isolation of such fractions from apple pomace and an evaluation of their hydrocolloidal functionality, by viscometry, was conducted by Walter *et al.* (1985).

Air-dried and milled apple pomace was subjected to mild alkaline degradation, yielding an α-cellulose fraction of approximately 26% of the untreated dry matter. Extraction by various aqueous solvents yielded water-dispersible, uronide fractions comprising 10-18% w/v, on a dry weight basis. These fibres had various viscosometric characteristics, depending on the extractant used. All the fibres produced had the potential to provide non-nutritive bulk to low fibre, constructed foods. It was also suggested that they may provide a high concentration of solid matter to an

aqueous food system without affecting its rheological properties significantly.

1.4.3 Apple Cellulose Gel

The development and characterisation of a cellulose gel from apple pomace was outlined in a paper by Walter *et al.* (1977). Cellulose was isolated from apple pomace in the form of a hydrogel, using dilute sodium hydroxide and hydrogen peroxide. Sodium hyroxide and hydrogen peroxide treatments have the effect of degrading lignin and other non-cellulosic constituents, leaving the cellulose fibres substantially intact.

The gel was characterised as a pseudo-plastic fluid with fluid properties similar to those of furcellan, guar and xanthan gums. Freeze-drying and milling of apple cellulose gel was found to impair its hydrophilic character, although the particles still showed a tendency to wet and redisperse.

1.4.4 Carbon Briquets

Low temperature pyrolysis as a method of recovering the fuel value of apple processing wastes was studied by Walter and Sherman (1976). Moist apple press cake was air-dried in a fume hood and then heated at 180^{o} $\pm 20^{o}C$ for 1.5 hours. A portion of the pyrolysate was further treated for conversion to granulated charcoal. This was achieved by suspending the pyrolysed presscake in a solution of sulphuric acid to produce

charred intermediates, which were easily separated from the aqueous medium. This mixture was then pyrolysed at 160-200^{o}C until smoking ceased (Walter and Sherman, 1975). It was found that the gross heats of combustion of apple charcoal briquets and apple pyrolysate were approximately 90% of that for a commercial barbeque briquet. The air-dried press cake contained approximately 62% compared to the commercial barbeque briquet.

At 180^{o}C, the sugar concentrated upon air-drying was transformed to thermoplastic caramels which had, with the addition of carbohydrate polymers, enough adhesive strength to bind the secondary reaction products and the ligno-cellulose fibres that escaped thermolysis. However, it is highly unlikely that the production of apple pyrolysate briquets would be economical due to the high energy requirements.

1.4.5 Apple Pomace as a Stock Feed

1) Cattle

Ruminant animals, with their ability to efficiently convert cellulosic substances to nutrients and waste, seem a likely target for apple pomace feeding trials.

A series of trials were conducted to determine the feasibility of supplementing apple pomace with nonprotein nitrogen (NPN) for winter feeding pregnant beef cows (Fontenot *et al.*, 1977). Feeding a combination of apple pomace with urea, biuret or a

combination of both lowered feed consumption and increased body weight losses in the cows, compared to supplementing corn silage with NPN or supplementing apple pomace with protein. Although, no signs of toxicosis were observed in the cows, feeding pomace and NPN in combination resulted in the birth of small, deformed dead or weak calves. Increasing the level of dietary energy did not prevent this occurrence, but feeding a small amount of coarse hay appeared to alleviate the effects.

Trials were conducted with gestating beef cows to determine whether the dietary addition of a commercial trace mineral premix, corn starch, or wheat straw would reduce the adverse effects of apple pomace-urea diets on weight gain and calving performance (Rumsey, 1979). Half the cows in each trial were orally administered copper. Dietary addition of a trace mineral premix or copper did not consistently improve cow weight gain or calving performance. Dietary starch improved weight gain but intensified the poor calving performance. In another series of trials gestating beef cows were fed apple pomace supplemented with cottonseed meal or urea and straw. Supplementing with cottonseed meal resulted in improved weight gain and normal calving performance. Supplementing with urea and straw resulted in poor weight gain but a normal calving performance. These results suggested that neither a lack of trace minerals nor ammonia toxicity was a major factor causing poor performance when gestating beef cattle were fed apple pomace-urea diets.

Although additional research was conducted on steers fed apple pomace-urea diets with respect to ruminal fermentation products, plasma ammonia (Rumsey, 1978), rumen microbial population, movement of ingesta from the rumen and water intake (Rumsey *et al.*, 1979), no causative factor was determined. However, it is apparent that apple pomace can be utilised as a cattle feed supplement if incorporated into a properly formulated diet.

Unfortunately, an apple pomace feed product may be of only limited value to the New Zealand ruminant industry due to the predominance of pasture feeding during virtually the whole year.

2) Pigs

The effects of dried apple pomace in swine rations on gowth rate, feed efficiency and carcass quality was studied by Bowden and Berry (1959). Experiments were conducted to test rations containing various levels of apple pomace. At pomace levels up to 20% no significant change occurred in daily gain, dressing percentage or carcass quality. Increasing the pomace level to 40% resulted in significantly slower growth, low dressing percentage, greater feed consumption per unit gain and a leaner carcass. In one series of trials, an estimated difference of 2 - 3% crude protein in the two ration feeds at each pomace level had no significant effect on any of the characteristics studied.

Snowden (1984) researched a least cost feed analysis

to determine the potential of dried apple pomace as a feed supplement for grower and breeder pigs. At that time, it was found that dried apple pomace with a value of NZ $180 per tonne had a possible 12.8% inclusion by weight in grower pig diets. The maximum inclusion rate in breeder pig diets was 12.5% at NZ $150 per tonne. A hypothetical demand curve for dried apple pomace in grower and breeder pig feeds gave a potential demand of 21,000 tonnes and 4,500 tonnes respectively, assuming that the product was fully utilised in all pig feeds throughout New Zealand. If all the apple pomace produced at present (27,000 tonnes/year) was dried to a microbially stable product of approximately 10% moisture, an annual production of about 6,000 tonnes/year would result. This suggests that the entire apple pomace waste stream could be utilised as a pig feed supplement if drying and distribution costs were not prohibitive.

1.5 FERMENTATION PROCESSES

Submerged fermentation, either by a direct one-stage process or by a multi-stage process, is generally considered the most efficient form of bioconversion of substrate. Present bioconversion schemes , however, are not applicable to all substrates under all conditions. This is true for apple pomace. An attractive alternative to submerged fermentation is solid state fermentation. Solid state fermentation is defined as microbial growth on solid particles in the absence of free fluid (Aidoo *et al.*, 1982). For the microbial transformation of marginally valuable

agricultural residues, solid state fermentation holds a number of advantages over the traditional submerged fermentation processes. Among the advantages are (Aidoo *et al.*, 1982; Durand and Chereau, 1988):

1) low level technology
2) reduced reactor volume per unit substrate converted
3) high productivity per unit volume of reactor
4) reduced energy requirement
5) low wastewater output
6) facility of product recovery

and 7) low capital investment.

Some disadvantages of solid state fermentation are (Aidoo *et al.*,1982; Durand and Chereau,1988):

1) difficulties in regulating fermentation parameters such as moisture, aeration, pH and heat removal
2) lack of knowledge concerning the physiology and growth mechanisms of micro-organisms on solid materials
3) types of micro-organisms are limited to those which can grow at reduced moisture levels

and 4) problems associated with scale up.

Although Durand and Chereau (1988) suggested that solid state fermentation would facilitate product recovery, no research on the recovery of fermentation products, especially secondary metabolites, generated

from solid materials has been found. Normal product recovery methods such as filtration, precipitation and distillation could be adversely affected by the particulate nature of the fermented material.

The following sections address some of the fermentation processes which have been proposed for the microbial transformation of apple pomace.

1.5.1 Acetone and Butanol

Currently, butanol is manufactured from ethylene and triethylaluminium and is used in the manufacture of lacquers, rayon, detergents, brake fluids and also finds use as an industrial solvent. Acetone is a by-product from the manufacture of phenol from cumene, or it can be manufactured by chemical reduction of iso-propanol. Acetone is used primarily as an industrial solvent (Linden *et al.*, 1985).

The acetone-butanol fermentation is a well known process which is awakening renewed interest due to the fluctuating values, strategic uncertainty and unsustainability of oil products. Traditional substrates used for acetone and butanol production by fermentation are a variety of starchy materials and molasses. With the cost of raw materials being a key factor in determining the viability of the acetone-butanol fermentation, the use of waste carbon sources may be beneficial (Jones and Woods, 1986).

The use of supplemented apple pomace as a substrate source for the production of acetone and butanol in a submerged fermentation system was studied by employing strains of *Clostridium acetobutylicum* and *Cl. butylicum* (Voget *et al.*,1985). Yields of butanol between 1.9% and 2.2% w/w of fresh apple pomace were reported, these results equating to reactor volume concentrations of approximately 13-15 g/l. The butanol:acetone ratio observed was 2:1 which is typical for acetone-butanol fermentations (Jones and Woods, 1986) on traditional substrates.

However, as is the case for all acetone-butanol fermentations, problems exist with low volumetric productivity, processing of biomass wastes, high water requirements, coproduct separation processes and butanol toxicity (Linden *et al.*, 1985).

1.5.2 Biodrying

The concept of biodrying involves a two-stage aerobic digestion and drying process combining high rate thermophilic aerobic digestion and low energy input drying. A predetermined amount of organic matter oxidation by thermophilic bacteria can be achieved by efficient management of substrate, water, energy and air for microbial use and drying (Jewell and Cummings, 1984). The exothermic heat of substrate oxidation provides the energy required to raise the temperature to thermophilic conditions and to preheat the drying air.

A biodryer feasibility study for the stabilisation and drying of apple pomace was conducted using a pilot scale prototype unit by Jewell and Cummings (1984). In a continuous feed study conducted over a 5 day period, a total mass and volume balance was maintained. In this system the pomace wet mass was reduced by 73% ; the volume of the pomace was reduced by 63% ; the biodegradable organics were reduced by 83% ; and approximately 1.3 times more energy was biologically produced than was consumed. The end product was dry, stable, odourless and remained quite high in digestible organics.

This process would undoubtedly produce a more environmentally sound product for waste disposal. In addition it would have some benefit in reducing the costs of transporting the apple pomace to landfill sites, due to the volumetric and mass reductions, but whether this would vindicate the necessary investment requires more study. Biodried apple pomace may find some use as a stock feed supplement but feeding and digestibility trials would first be required. If found to be unacceptable as a feed supplement the dry material may find application in the horticultural sector as a soil conditioning compost.

1.5.3 Anaerobic Digestion

A variety of technologies have been developed for energy production from biological fuels and these include combustion, fermentation, gasification and pyrolysis. Anaerobic digestion is an attractive

option, yielding a high value natural gas substitute as well as a stable residue which may have some market potential.

Using bench scale mesophilic (35°C) and thermophilic (55°C) reactors and a pilot scale thermophilic reactor with an apple pomace substrate, Jewell and Cummings (1984) achieved an average biodegradability of 76.3% at a loading rate of 5 g/l/day. This resulted in a biogas production of 3 v/v-d (volume of gas per volume of reactor per day). The methane content of the gas effluent was 60%.

In a full scale system Etheridge and Jewell (1988) used a flexible liner digester for the anaerobic digestion of fresh and ensiled pomace. It was found that the biogas potential of ensiled pomace was similar to fresh pomace, demonstrating that ensiling of apple pomace is feasible at full scale. Little difference between mesophilic and thermophilic operations, in relation to biogas production and removal efficiency, was noted except that stable mesophilic operation was difficult to maintain. Mesophilic systems were discovered to fail at organic loading rates greater than approximately 3.6 g/l/day. Run under thermophilic conditions at 55°C a 90% conversion of biodegradable volatile solids at a loading rate of 5 g/l/day was achieved, with a concomitant biogas production of 1.9 v/v-d.

1.5.4 Citric Acid

For the last 60 years citric acid has been produced by fermentation of carbohydrates. Originally, the surface process utilising the fungus *Aspergillus niger* was used. Latterly, submerged fermentations of beet or cane molasses or glucose syrups by *A. niger* have been introduced. A recent development has been the replacement of *A. niger* by strains of yeast which exhibit higher productivity and are less sensitive to variations in crude carbohydrate media (Milsom and Meers, 1985).

An evaluation of the suitability of apple pomace as a substrate source for citric acid production was conducted by Hang and Woodams (1984). In this study five strains of *A. niger* were used for the production of citric acid from apple pomace in a solid state fermentation. *A. niger* NRRL567 was found to produce the greatest amount of citric acid in the presence of 4% methanol. A yield of 88%, based on the amount of sugar consumed, was achieved. This compares favourably with yields of citric acid from a sucrose or molasses medium impregnated in a sugarcane bagasse carrier (Kapoor *et al.*, 1982).

A subsequent report by Hang and Woodams (1987) determined the effect of apple pomace moisture on fungal production of citric acid in a solid state system. It was found that the stimulatory effect of methanol on fungal production of citric acid decreased markedly as the pomace moisture decreased. Conversely, the citric acid production in the absence of methanol

addition increased as the pomace moisture level decreased.

The recovery of citric acid from the solid state fermentation would require a solvent extraction step, to remove the acid produced from the fibrous medium. To what extent this would effect downstream processing has not been addressed.

1.5.5 Ethanol

A solid state fermentation system for the production of ethanol from apple pomace using *Saccharomyces cerevisiae* was devised by Hang *et al.*, (1982). Pomace inoculated without pasteurisation or autoclaving with the yeast strain fermented rapidly at 30°C and had an ethanol content of more than 4% in 24 hours. It was shown that temperature had a marked influence on the rate of ethanol production. The fermentation times required for maximal production of alcohol at 30°C, room temperature (22-25°C) and 15°C were 24, 48, and 96 hours respectively. Of the alcohols analysed, ethanol was present in the highest concentration with the other alcohols being produced in much smaller quantities. The separation of alcohol from the pomace was achieved using a Buchi rotary vacuum evaporator. The efficiency of this operation ranged from 92-99% with an ethanol concentration in the final condensate as high as 13% v/v.

The protein content of the spent apple pomace was found to have increased from 4% to 9%, on a dry weight

basis. Hang *et al.* (1982) suggested that it may be used as a stock feed supplement although the still minimal protein content and combined drying costs required to form a microbially stable product would possibly make this option unfeasible.

The production of ethanol by solid state fermentation of apple pomace using the yeasts *Saccharomyces cerevisiae*, *Sacch. diastaticus*, *Pichia fermentans*, *Candida utilis* and *C. tropicalis* was conducted by Gupta *et al.* (1989). *Sacch. cerevisiae* and *Sacch. diastaticus* were found to have the greatest alcohol yields (2.6 and 2.8 g/100 g, respectively) of the five yeasts used. The effect of nitrogen, phosphate and trace element supplementation was compared in *Sacch. cerevisiae* and *Sacch. diastaticus* on the basis of the fermentation efficiency of apple pomace. The addition of various nitrogenous sources, phosphates or trace elements resulted in an improved fermentation efficiency for both yeasts.

Of the nitrogenous sources added ammonium sulphate effected the greatest increase in fermentation efficiency at an inclusion rate of 0.2% w/v. The addition of dipotassium hydrogen phosphate (0.2% w/v) resulted in the greatest increase in fermentation efficiency of the phosphate sources added. Although a greater increase was achieved with ammonium dihydrogen phosphate supplementation, this increase would have been largely due to the additional nitrogen content. The addition of individual trace elements (0.002% w/v) was found to improve fermentation efficiency, with the addition of zinc sulphate resulting in the greatest

addition of zinc sulphate resulting in the greatest increase.

The greatest alcohol yield obtained (Gupta *et al.*, 1989) was 5.7% using the yeast *Sacch. diastaticus* for the solid state fermentation of apple pomace supplemented with 0.2% w/v ammonium dihydrogen phosphate.

Industrial alcohol is produced from whey in New Zealand at Reporoa, Tirau and Clandeboye (Mawson, 1987). Potable ethanol is supplied by the New Zealand Distillery Co. Ltd., Edgecumbe, but limited quantities are also produced at Reporoa and Clandeboye. These four distilleries have a combined annual output of approximately 11 million litres absolute alcohol (Mawson, personnal communication) and supply both the N.Z. and limited export markets. As a result, any future for the production of ethanol from apple pomace in N. Z. would depend on its competitiveness with the established whey ethanol process.

1.5.6 Pectinases

The possible use of apple pomace for the production of pectinases in a solid state culture using an *Aspergillus foetidus* strain was investigated by Hours *et al.* (1988).

Using small scale solid state cultures, pectinase production reached viscosometric enzyme activities as high as 1062 U/g. These results were obtained after 48

fermentation, the maximum value achieved in this case was more than seven times higher than the values reported per millilitre by Tuttobello and Mill (1961). The period for obtaining the maximum enzyme levels was also reduced from 120 hours to 48 hours. The pool of pectinases obtained was characterised as containing endopectinylase and a polygalacturonase. The presence of amylases, cellulases and hemicellulases was also shown, but no proteases or lipases were detected.

Although apple pomace appears to be an adequate raw material for pectinases production, problems associated with the extraction of the enzymic pool have yet to be addressed.

1.5.7 Microbial Biomass Product

Present day processes for producing yeasts for food and feed protein originated in Germany during World War I and II, where both *Saccharomyces cerevisiae* and *Candida utilis* were grown on molasses and sulphite-waste liquor, respectively (Litchfield, 1980). During the last two decades, numerous processes have been developed for producing cells of micro-organisms for use as protein sources for human food or animal feed. It appears that food grade single cell protein (SCP) products may not enter the human nutritional markets to any great extent in the short term due to the relatively lower costs of alternative proteins from plant and animal sources, and the reqirements of regulatory agencies for extensive safety studies (Litchfield, 1985). However, Rank, Hovis and McDougal

have successfully marketed a fungal protein meat substitute filling for pastries recently. Also, microbial protein products for animal feed application are quite competitive with conventional plant and animal sources. A large number of micro-organisms and substrates have been analysed for their suitability for microbial protein production. Yeast protein is largely limited to the production of *Candida utilis*, with smaller quantities of protein produced from the *Saccharomyces cerevisiae* generated as a waste stream from wine and beer fermentations. British Petroleum and Liquichimica plants did produce a *Candida* sp. from purified n-alkanes (Kanazawa, 1975) and a plant in Oregon, U.S.A. produces *C. utilis* from sulfite waste liquor (Anderson *et al.*, 1974). *Kluyveromyces fragilis* has been produced from cheese whey in Wisconsin, U.S.A. (Bernstein *et al.*, 1977). With the volatility of petrochemical prices and the increasing values of traditional starch substrates much attention is now being focused on waste materials as carbon sources, including apple pomace.

Apple pomace has generally been regarded as a poor animal feed supplement due to its low protein content. However, the chemical composition of pomace suggests that it may be used for the production of a protein-enriched microbial biomass product (Hours *et al.*, 1985), a term used to denote the complex mixture of substrate source and micro-organisms obtained by the fermentation of undefined agricultural wastes. Studies on the formation of microbial biomass products from a number of substrate sources including annual ryegrass straw (Han *et al.*, 1978), rice straw (Han and

Anderson, 1974), wheat straw (Viesturs *et al*, 1981; Laukevics *et al.*, 1984), sugar-beet (Cochet *et al.*, 1988; Durand and Chereau, 1988) and apple pomace (Hours *et al.*, 1985; Hang, 1988) have been conducted using bacterial, yeast and fungal cultures. With respect to apple pomace, both submerged and solid state fermentation trials have been conducted, under various conditions and using different micro-organisms, to produce a protein enriched feed.

Small scale batch and fed-batch submerged fermentation processes using *Saccharomycopsis* (now *Yarrowia*) *lipolytica* and *Trichoderma reesei* in single and mixed cultures were analysed by Hours *et al.* (1985).

Batch fermentations in 200 ml Erlenmeyer flasks using a single culture of *Y. lipolytica* on a supplemented pomace substrate attained a protein yield (protein produced/sugar consumed) value of 0.45 after 34.6 hours. This corresponded to a protein content, on a dry weight basis, in the final product of 13%.

A 4-l fermenter trial using *Y. lipolytica* and pomace diluted with 33% water produced a protein content of 12.5% with the advantage of reducing the time of the process by approximately 50%. This was explained by the greater aeration efficiency at fermenter scale and it was therefore considered that the kinetics of the operation were controlled by the oxygen transfer rate. However, it seems that no consideration was given to the differences in fermentation procedure whereby pH in the Erlenmeyer flask was maintained using 1 M NaOH whilst pH in the fermenter was adjusted using 7% v/v

NH_4OH. It is highly likely that the nitrogen addition in the fermenter trials had a major bearing on the reduction in maximum protein production time.

Preliminary experiments using *T. reesei* in Erlenmeyer flasks (Hours *et al.*, 1985) revealed that the normal flora in the apple pomace competed strongly with the fungi, resulting in a decreased yield. For this reason, a medium containing apple pomace treated with sulphuric acid at 100°C for 2 hours was used. The combination of acid and heat treatment reduced the content of the normal flora, decreased the viscosity of the medium and produced a partial hydrolysis of the pectic substances and crude fibre, increasing the amount of substrate available for growth by 20%.

Experiments carried out in Erlenmeyer flasks with *T. reesei* using the acid/temperature-treated media showed that the protein level of the fermented material at 48 hours corresponded to 15% on a dry weight basis. As no trials were conducted on the acid/temperature-treated pomace using *Y. lipolytica*, no accurate comparison between the yeast and fungal protein production could be made for this medium.

Although *T. reesei* was found to have a lower growth rate than *Y. lipolytica* the pectolytic and cellulolytic activities of the fungus were considered beneficial in further reducing pectic substances and increasing soluble sugars in the treated pomace. As a result, fed-batch fermentation trials using a mixed culture of *T. reesei* and *Y. lipolytica* were conducted in order to increase the amount of dry matter at the

end of the process. A protein content of 15.6% was achieved which equated to a volumetric value of 15 g/l, which was double the values obtained in other experiments.

A study to improve the nutritional value of apple pomace by solid state fermentation with the food yeast *Candida utilis* was conducted by Hang (1988). Contrary to results obtained by Hours *et al.* (1985), *C. utilis* was shown to grow well on an ammonium sulphate-supplemented pomace substrate and significantly improved its nutritional value. Yeast growth resulted in a protein content of 15% on a dry weight basis. Significant increases in niacin and pantothenic acid, and small increases in both riboflavin and thiamine were observed. The enhancing effect of yeast fermentation on protein and vitamin yields was found to be dependent on the amount of ammonium sulphate added, up to a concentration of 1% w/w.

1.5.8 Summary

With the production of apple pomace wastes being such a disposal problem, research to find a constructive solution is necessary. Improved disposal methods and treatments that would be less harmful to the environment are plentiful but costly. However, apple pomace has compositional characteristics which suggest that some profitable use could be found for it. Research into possible productive uses for apple

pomace has been going on for the last three decades and yet none of the research has progessed from the laboratory to the factory, in N.Z. at least. Whether this is due to the lack of cost effectiveness of the processes or an unwillingness on the part of the apple industry to invest in new technologies due to the financial risks involved is not always clear. However, with the increasing environmental concern being shown by the populace, opposition to the dumping of apple pomace may also increase, forcing the apple industries to find some other method of disposal or usage.

A significant amount of research has been conducted into the processing of waste apple pomace from the development of human food products, ie. edible fibres, press cake powder and cellulose gels, to animal feed products, fermented and unfermented, to fuels, ie. carbon briquets, anaerobic digestion (biogas), ethanol production and biodrying, to the production of valuable secondary products, ie. acetone, butanol, citric acid and pectinases. Of the options covered in this literature the most promising treatments would appear to be anaerobic digestion, citric acid production and the use of pomace as an animal feed in either a fermented or unfermented, dried state. The following research to be described addresses aspects of producing a nutritionally improved apple pomace stockfeed by solid state fermentation using yeasts.