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PINEAPPLE WASTE - A NOVEL SUBSTRATE FOR CITRIC ACID PRODUCTION BY SOLID-STATE FERMENTATION

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SUMMARY

Citric acid production in solid-state fermentation by *Aspergillus foetidus* ACM3996 was better on pineapple waste than on apple pomace, wheat bran or rice bran. The highest citric acid content achieved on pineapple waste was 16.1 g per 100 g dried pineapple waste, with a moisture content of 70% and in the presence of 3% methanol. This represents a yield of 62.4% based on the sugar consumed.

INTRODUCTION

Pineapple peel is a byproduct from the extraction of pineapple juice, and constitutes about 30% by weight of the original fruit. Approximately 400 000 tonnes of pineapple peel are produced annually in Australia. Most pineapple peel is used for animal feed. However, pineapple peel should be suitable for citric acid production, since other wastes containing sucrose, such as cane and beet molasses have long been used (Kapoor et al., 1982).

Extraction of the sugars from pineapple waste for a liquid fermentation process would lead to a relatively dilute medium. This would decrease citric acid yields and favour oxalic acid production (Kovats, 1960), significantly increasing recovery costs. Solid-state fermentation (SSF) has been used successfully in citric acid production on other solid substrates such as apple pomace, grape pomace, kiwifruit and wheat bran (Hang and Woodams 1984, 1985, 1986a, 1986b, 1987; Hang et al., 1987; Shankaranand & Lonsane, 1992).

This study evaluates the potential of pineapple waste as a substrate for citric acid production by solid-state fermentation using the very productive new species *Aspergillus foetidus* ACM 3996 described by Chen (1994). The time of harvest, moisture content and methanol content are optimized.

METHODS AND MATERIALS

Wet pineapple waste and apple pomace (from the Golden Circle Cannery, Brisbane) were dried at 52°C for 60 h to a moisture content of 7%, then ground to the desired size in a hammer mill.

Spores were suspended in 3 ml of sterile water added to each PDA slant of *Aspergillus foetidus* ACM 3996. 10 g of dried substrate was added to each flask and water was added to achieve the desired water content. Flasks were autoclaved at 121°C for 15 min, cooled, inoculated with 0.2 ml spore suspension containing about 1×10^7 spores/ml, and then incubated at 30°C.

Samples were mixed with 10 volumes of deionized water (based on the initial weight of moist solids), shaken on a rotary shaker for 60 minutes, then filtered through Whatman No. 1 filter paper. Solids were washed with distilled water several times to extract the sugars, and the extracts were combined and made up to 500 ml.

Moisture content samples were dried to constant weight at 105°C. Citric acid was measured with a Waters HPLC fitted with a Biorad HPX-87 column and eluted with an aqueous solution of sulfuric acid at 0.6 ml/min. Sugars were measured with a Waters HPLC with a Shodex S-801/S column eluted with water at 0.5 ml/min. Both HPLC units had refractive index detectors. Citric acid and sugar concentrations are expressed as g per 100 g of initial dried pineapple waste, and the percentage yield represents g of citric acid per 100 g of sugar consumed.

RESULTS & DISCUSSION

Comparison of substrates

Citric acid production by *A. foetidus* was best on pineapple waste, with a citric acid content of 9.9 g per 100 g of initial dry pineapple waste achieved in 4 days (Table 1), representing a yield of 39.7% based on the sugar consumed. In SSF of various substrates by various strains of *Aspergillus niger* results have included: 9.2 g citric acid per 100 g of grape pomace at a yield of 60% based on the sugar consumed (Hang & Woodams, 1985); 21.2 g per 100 g kiwifruit peel at a yield of 60% (Hang et al., 1987); 6.5 g per 100 g apple pomace at a yield of 88% (Hang & Woodams, 1984); 7.2 g per 100 g wheat bran, at a yield of 33% (Shankaranand & Lonsane, 1992); 15.0 g per 100 g of coffee husk at a yield of 82% (Shankaranand & Lonsane, 1994); and 17.4 g per 100 g of sugarcane pressmud plus sucrose at a yield of 80% (Shankaranand & Lonsane, 1993). Pineapple waste shows promise as a substrate for citric acid production if the yield can be improved.

The production of citric acid on pineapple waste by *A. foetidus* was investigated in more detail. Sugar consumption was very rapid for the first 4 days, and the maximal citric acid content of 11.0 g per 100 g of initial dry pineapple waste was achieved on the fourth day (Fig. 1). Although sugar consumption then continued for several days at a slower rate, the citric acid content actually fell slightly.

Table 1. Citric acid production by *A. foetidus* ACM 3996 from various substrates after 4 days solid-state fermentation at 30°C

Substrate	Initial moisture (%)	Initial sugar (g/100 g)	Residual sugar (g/100 g)	Citric acid (g/100 g)	Yield (%)
Pineapple	65	25.6	0.5	9.9	39.7
Apple	65	39.8	9.9	4.7	15.8
Wheat bran	65	5.5	0.3	0.8	15.0
Rice bran	50	12.9	0.3	2.8	22.8

Effect of moisture content

The time at which maximal citric acid production is achieved varies with the fermentation conditions (Kapoor et al., 1982). Therefore, citric acid contents were measured daily from the second to the sixth day of fermentation. The highest citric acid concentration of 11.7 g/100 g was achieved in 3 days for a moisture content of 70% (Table 2). However, reasonably high concentrations (above 10.5 g/100 g) were obtained from 65 to 80% moisture and with harvest times of 3 or 4 days.

The optimum moisture content for citric acid production from apple pomace was 45% in the absence of methanol, but 65% in the presence of 3% methanol (Hang & Woodams, 1987). In the absence of methanol the yield of citric acid from kiwifruit peel increased with decreasing moisture content, but in the presence of methanol the citric acid yield increased with increasing moisture content (Hang et al., 1987). Since the presence of methanol affects the response to environmental conditions, it was necessary to determine the effect of methanol prior to any further optimization. A moisture content of 70% was used for this study.

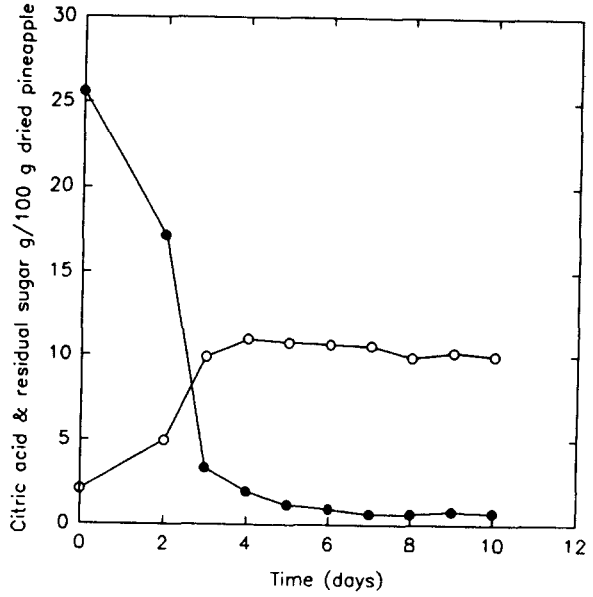

Fig. 1. Citric acid production from pineapple waste by *A. foetidus* ACM 3996, grown at 30°C, and 65% initial moisture (o) citric acid; (•) residual sugar

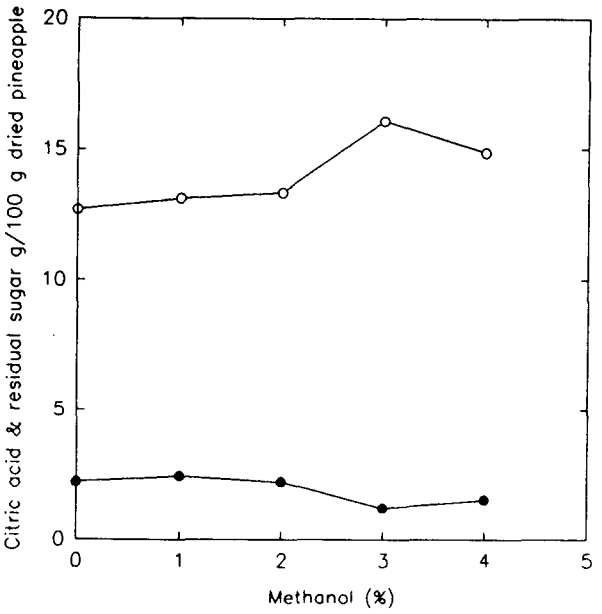

Fig. 2. Effect of methanol on citric acid production from pineapple waste by *A. foetidus* ACM 3996 after 4 days at 30°C with 70% initial moisture (o) citric acid; (•) residual sugar

Table 2. Effect of substrate moisture content on production of citric acid by *A. foetidus* ACM 3996 in solid-state fermentation of pineapple waste

Time (days)	Moisture content 55%	60%	65%	70%	75%	80%	85%
	Citric acid content (g per 100 g initial dry substrate)						
2	3.9	4.0	8.8	9.9	10.0	10.7	10.5
3	3.3	10.0	10.7	11.6	11.3	11.0	9.6
4	4.9	10.4	10.0	10.5	10.9	10.7	9.8
5	8.3	10.6	10.7	10.5	10.4	10.0	9.7
6	9.3	10.3	10.2	10.7	10.4	10.1	9.6

Effect of methanol

Methanol contents up to 3% (v/w) increase the citric acid production within 4 days fermentation by *A. foetidus* (Fig. 2). With 3% methanol a citric acid content of 16.1 g/100 g dried pineapple waste was obtained, representing 62.4% conversion based on total sugar consumed. This stimulation by methanol is similar to that obtained for *A. niger* during SSF of apple pomace (Hang & Woodams 1984, 1985, 1986a). The increase in citric acid yield with methanol is a general phenomenon and is commonly used in citric acid production (Kapoor et al., 1982). Methanol is not assimilated. Its role may be connected with an altered permeability of the cell membrane, allowing greater excretion of citric acid (Kapoor et al., 1982).

CONCLUSION

Solid-state fermentation of pineapple waste by *A. foetidus* in the presence of 3% methanol yielded 161 g of citric acid per kg of dried pineapple waste in four days. This yield was 62.4% based on the amount of sugar consumed. The use of pineapple waste for citric acid production may have the combined benefit of utilizing a low value waste material while producing a commercially valuable product.

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