J Food Sci Technol (July–August 2010) 47(4):458–460
DOI: 10.1007/s13197-010-0077-2

ORIGINAL ARTICLE

Citric acid production by *Aspergillus niger* van. Tieghem MTCC 281 using waste apple pomace as a substrate

Dinesh Kumar · Rachna Verma · Bhalla T. C.

Revised: 25 August 2009 / Accepted: 2 September 2009

Abstract A solid state fermentation process was tried for the production of citric acid from apple pomace left after juice extraction using *Aspergillus niger* van. Tieghem MTCC 281 spores as inoculum (36.8×10^4 spores/100 g of pomace). The yield of citric acid was optimized by varying the amount of methanol (1–5% v/w), temperature (25–35°C) and time of incubation (1–7 days) for fermentation process. Optimum yield of citric acid (4.6 g/100 g of pomace) was recorded with 4% (v/w) methanol after 5 days of incubation at 30°C.

Keywords Citric acid production · Waste apple pomace · *Aspergillus niger* van. Tieghem MTCC 281

Kumar D. · Verma R. · Bhalla T. C.
[1]Abhilashi Institute of Life Sciences, Ner Chowk, Mandi - 175 008, India
[2]Department of Biotechnology, Himachal Pradesh University, Shimla - 171 005, India

Kumar D. (✉)
E-mail: chatantadk@yahoo.com

Introduction

The wide spread presence of citric acid in the animal and plant kingdom is an assurance of its non-toxic nature and it has long been used in the food, beverage, pharmaceutical and cosmetic industries (Vandenberghe et al. 2004). Citric acid is commercially produced by fungal submerged fermentation of molasses with white rot fungus (*Aspergillus niger*) due to its high productivity at low pH without secretion of toxic by-products (Kapoor et al. 1982, Pallares et al. 1996, Jianlong et al. 2000). Solid state fermentation (SSF) offers numerous advantages and has lower energy requirements, produces less waste water and is environment-friendly as it resolves the problem of solid waste disposal (Hang et al. 1982).

There are many reports on efficient utilization of agro-industrial residues and by-products for citric acid production using less expensive substrates, such as apple and grape pomace, carrot waste, orange and pineapple waste, cassava bagasse, coffee husk, kiwifruit peel, rice and wheat bran (Hang and Woodams 1986a, Grewal and Kalara 1995, Lu et al. 1998, Soccol and Vandenberghe 2003). These residues are very well adapted to SSF due to their cellulosic and starchy nature. The substrate is saturated to about 70% moisture and inoculated with the microorganism. The pH of the process is normally adjusted to 4.5–6.0 and incubated at 28–30°C depending upon the microorganism used (Vandenberghe et al. 1999).

Apple pomace is the residue left after juice extraction and constitutes about 25–35% of the weight of fresh fruit. It contains 12.3% fermentable sugar (Hang and Woodams 1986b) and is rich in carbohydrates, but its protein content is very low (Bomben et al. 1971). Apple pomace contains 85% carbohydrates (76% natural sugar and 9% uronic acid) and 15% w/v protein (Reid et al. 1999). Water soluble components of apple pomace are composed of mono-oligosaccharide and water soluble polysaccharide, and water insoluble components including pectic substances,

hemicelluloses and cellulose and the total composition ratio is 56:1:17:15:11 in order (Noro et al. 2006). At present, most of the pomace left after juice extraction is dumped on land despite the increasing disposal problem and efforts for by-product utilization (Hang et al. 1982). Because of its physical nature, apple pomace is not readily amenable to submerged fermentation and it is necessary to dilute the pomace with water, and yield of citric acid from such a dilute pomace mash is too small to recover economically. In the present study a SSF method is reported for the production of citric acid from apple pomace left after juice extraction.

Samples of apple (*Malus domestica*) pomace varieties, 'Royal Delicious' and 'Red Delicious' were obtained from fruit processing unit of Himachal Pradesh Horticultural Produce and Processing Marketing Corporation, Parwanoo, India, and stored at –20°C for further investigation. Before use, the pomace was grounded with a mixer grinder to obtain fine size (1–2 mm) particles. The citric acid producing strain *Aspergillus niger* Van Tieghem MTCC 281 was obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The culture was grown on Czapek Dox Agar slants at 30°C for 7 days. A spore inoculum was prepared by adding 3 ml of sterile distilled water to the slants and shaking vigorously for 1 min.

Solid state fermentation (SSF): SSF of pomace was conducted as follows: 100 g of apple pomace was taken into 1000 ml Erlenmeyer flasks. Each flask was inoculated with $36.8x10^4$ spores/100 g of pomace and incubated at 30°C with varying concentrations of methanol (1–5% v/w) before fermentation for 5 days. Citric acid production was studied over a period of 7 days at incubation temperatures varying from 25 to 35°C.

Analytical methods: At the completion of fermentation run, the fermented materials were extracted with water (1:1 ratio) and extracts were analyzed for residual sugar by DNS method (Miller 1959) and citric acid content by the colorimetric method of Vandenberghe et al. (2004).

All experiments were carried out in 3 replicates. Increasing the concentration of methanol up to 4% resulted in a marked increase in citric acid production. Similar results were observed earlier with *A. niger* NRRL 567 for requirement of methanol in citric acid production (Hang and Woodams 1986a). However, optimum citric acid production was observed with 2 and 3% methanol respectively, with kiwifruit peel (Hang et al. 1987) and pineapple waste

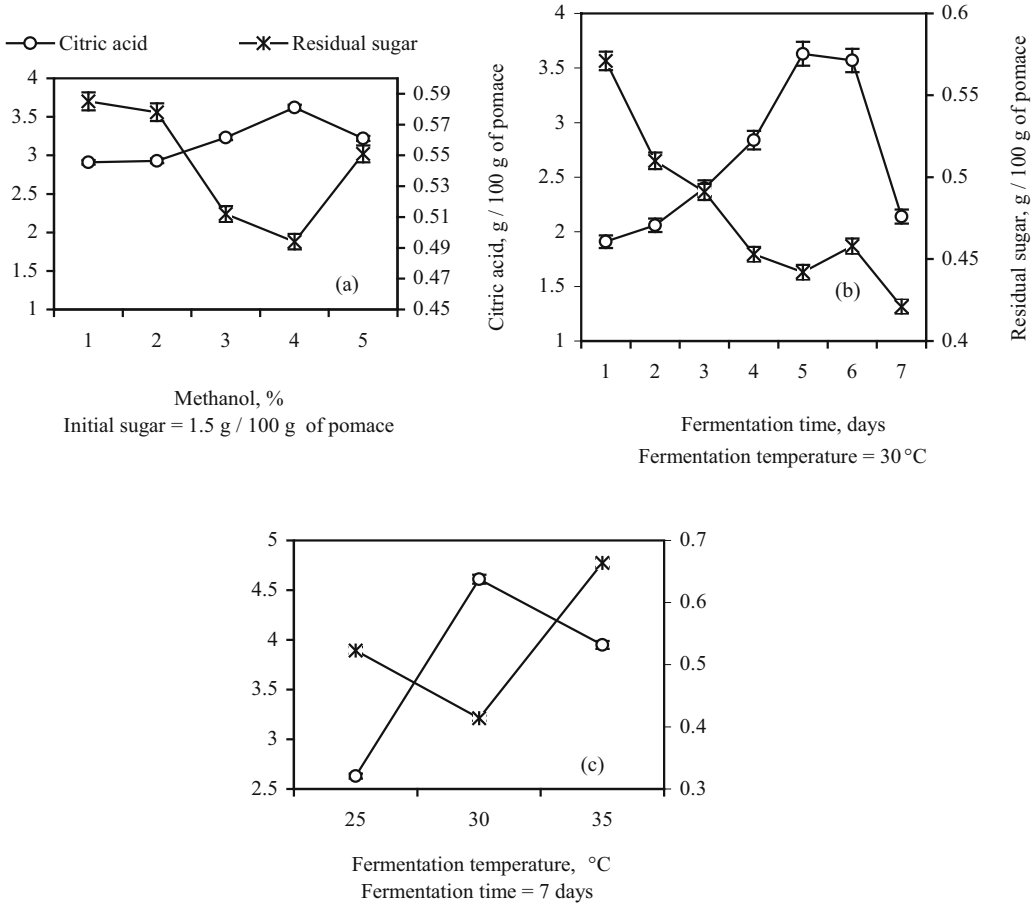

Fig. 1 Effect of methanol concentrations (a), fermentation time (b) and temperature (c) on production of citric acid by solid state fermentation using apple pomace waste as substrate and *Aspergillus niger* van. Tieghem MTCC 281 spores (n = 3)

(Tran et al. 1998). The influence of methanol in increasing citric acid production appears to be a general phenomenon with the strains of *A. niger* and is used frequently in the commercial productions (Kapoor et al. 1982).

The production of citric acid approximately paralleled the consumption of available sugar. Citric acid production with 4% methanol increased rapidly up to 5 days. Kapoor et al. (1982) also reported 7–10 days time requirement for maximum citric acid production with submerged fermentation of molasses. But, higher yield of citric acid is achieved in less than 7 days of spore incubation and this might be due to the increase of the product concentration in the fermentation medium (Hang and Woodams 1986b, Vergano et al. 1996).

The optimum fermentation temperature for production of citric acid by *A. niger* van Tieghem MTCC 281, grown on apple pomace in the presence of 4% methanol, was 30°C. The mould produced only a small amount of citric acid at 25°C in 5 days. The sporulation of *A. niger*, however, was more marked at 35°C than at lower temperatures. In commercial citric acid production the fermentation temperature is typically between 25–30°C, although slightly higher temperature can be employed (Friesen et al. 1999). Optimum temperature of 30°C is also reported in SSF of pineapple waste (Tran et al. 1998) and kiwifruit peel (Hang et al. 1987) for citric acid production.

In conclusion a solid state fermentation process is optimized for citric acid production by *A. niger* van Tieghem MTCC 281 using waste apple pomace as a substrate. This process yielded as high as 4.6 g citric acid per 100 g of apple pomace in the presence of 4% methanol in 5 days at 30°C. Use of apple pomace as a substrate might have economic value in the production of commercially valuable citric acid and in handling waste disposal problem.

Acknowledgements Authors are thankful to the Abhilashi Educational Society, Mandi, India and Himachal Pradesh University, Shimla, India respectively for the financial support and technical help in compilation of the present work.

References

Bomben JL, Gaudagni DC, Harris JG (1971) Apple-flavored thickener from apple peel. Food Technol 25:1108–1117

Friesen DT, Babcock WC, Brose DJ, Chambers AR (1999) Recovery of citric acid from fermentation beer for using supported liquid membranes. Membrane Sci 56:127–141

Grewal HS, Kalara KL (1995) Fungal production of citric acid. Biotechnol Adv 13: 209–234

Hang YD, Lee CY, Woodams EE (1982) A solid state fermentation system for production of ethanol from apple pomace. J Food Sci 47:1851–1852

Hang YD, Luh BS, Woodams EE (1987) Microbial production of citric acid by solid state fermentation of Kiwifruit peel. J Food Sci 52:226–227

Hang YD, Woodams EE (1986a) Utilization of grape pomace for citric acid production by solid state fermentation. Am J Enol Vitic 37:141–142

Hang YD, Woodams EE (1986b) Solid state fermentation of apple pomace for citric acid production. MIRCEN J 2: 283–287

Jianlong W, Xianghua W, Ding Z (2000) Production of citric acid from molasses integrated with *in situ* product separation by ion exchange resin adsorption. Biores Technol 75:231–234

Kapoor KK, Chaudhary K, Tauro P (1982) Citric acid. In: Presscott and Dunn's Industrial Microbiology. Reed G (Ed), AVI Westport, Connecticut, p 709–747

Lu MY, Maddoz IS, Brooks JD (1998) Application of multilayer packed bed reactor to citric acid production in solid state fermentation using *Aspergillus niger.* Process Biochem 33: 117–123

Miller JL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Biochem 31:426–428

Noro S, Takahashi T, Ichita J, Muranaka Y, Kato Y (2006) Sugar composition of apple pulp polysaccharides and their enzymatic hydrolysis. Trans Mater Res Soc Japan 31:973–976

Pallares J, Rodriguez S, Sanroman A (1996) Citric acid production in submerged and solid state culture of *Aspergillus niger*. Bioprocess Eng 15:31–33

Reid S, Sims IM, Melton LD, Gane AM (1999) Characterization of extracellular polysaccharides from suspension cultures of apple (*Malus domestica*). Carbohydrate Polym 39:369–379

Soccol CR, Vandenberghe LPS (2003) Over-view of applied solid state fermentation in Brazil. Biochem Eng 13:205–218

Tran CT, Sly LI, Mitchell DA (1998) Selection of a strain of *Aspergillus* for the production of citric acid from pineapple waste in solid-state fermentation. World J Microbiol Biotechnol 14: 399–404

Vandenberghe LPS, Soccol CR, Panday A, Lebeaulde JM (1999) Microbial production of citric acid: Review. Braz Arch Biol Technol 42:263–276

Vandenberghe LPS, Soccol CR, Prado FC, Pandey A (2004) Comparision of citric acid production by solid state fermentation in flask, column, tray and drum bioreactor. Appl Biochem Biotechnol 118(3):293–303

Vergano MGF, Soria MA, Kerber NL (1996) Influence of inoculum preparation on citric acid production by *Aspergillus niger.* World J Microbiol Biotechnol 12(6):655–656