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Versatility of Cassava Feedstock for Lactic Acid Production

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Abstract

Various forms of cassava materials including dried chips (containing 73% starch, wet basis) and fresh roots (containing 26% starch, wet basis) were used as inexpensive feedstocks for lactic acid production by homofermentative *Lactobacillus rhamnosus* DM3. The starch accumulated in cassava materials was first liquefied (300 g dry solid l^{-1}) by α-amylase and subsequently subjected to Simultaneous Saccharification and Fermentation (SSF) process (150 g dry solid l^{-1}, working volume of 2.5l) with glucoamylase and *L. rhamnosus* seed culture (5%v/v). Under the controlled condition (40°C and pH 6.0), the fermentation outputs of lactic acid production from dried chips and fresh roots were comparable to the use of starch (lactic acid concentrations were 145, 159 and 141 g l^{-1}; the acid productivities were 4.03, 3.3 and 4.69 g $l^{-1}h^{-1}$ and the yields were 99, 110 and 96% for dried chips, fresh roots and starch, respectively. The expanded uses of cassava feedstock as dried chips and fresh roots can synergetically promote the growth in cassava demand as well as the cost effectiveness in lactic acid production.

***Key words**:* cassava starch, dry chips, fresh roots, lactic acid, *lactobacillus rhamnosus*

Introduction

Cassava (*Manihot esculenta* Crantz) is the third most important crop in Thailand. The annual root productivity in Thailand is about 18 to 20 million tons fresh root and still continuously increases under the supportive policy of the government to adequately meet the industrial demand. The country has demonstrated the importance of cassava as more than a subsistence crop to be an economic crop, and has developed a large and complex industrial system for processing and marketing of this crop.

Lactic acid is widely used in food, cosmetic, pharmaceutical and chemical industries and has received an increasing attention for used being as feedstock in the production of biodegradable poly lactic acid (Datta et al. 1995; Sreenath et al. 2001; Tokiwa and Jarerat 2004). The worldwide lactic acid production is expected to reach more than 500,000 tones in 2010 (Bochoux et al. 2006). Lactic acid can be synthesized by a chemical process. However, the production by bioprocess with lactic acid bacteria fermentation is of great interest as it involves the utilization of renewable feedstock. Sugars are commonly used and starches from different botanical sources, e.g. corn, wheat, rice, potato, sorghum and cassava are alternatively used. Instead of using extracted and purified starch, low-priced primary products of cassava, i.e. fresh roots and dried chips having high starch contents can also be applied to reduce the overall production cost of lactic acid. The use of dried chips and fresh roots for lactic acid production was then demonstrated in this work.

Materials and Methods

Microorganism and media

Homofermentative *Lactobacillus rhamnosus* DM3, a new strain isolated from Thai fermented pork that producing mainly L (+)-lactic acid (Timbuntam et al., 2008), was grown in pre-culture medium, containing 5 g yeast extract, 10 g peptone, 20 g glucose, 1 ml Tween 80, 0.2 g $MgSO_4.7H_2O$, 0.005 g $MnSO_4.4H_2O$ and 5 g sodium acetate per 1 liter of distilled water. Commercial enzymes including α-amylase (Liquazyme Supra, Novozymes, Denmark), and glucoamylase (OPTIDEX L-400, Genencor International Inc. U.S.A.) were employed for starch hydrolysis. Cassava starch was obtained commercially from Sanguan Wongse Industries Co., Ltd (Thailand). Dried chips and fresh roots were obtained locally. The starch contents of all materials were quantified according to Polarimetric method-the European Economic Communities, EC 79, 1999. Fish wastes were kindly supported by I.S.A Value Co., Ltd., Thailand and hydrolyzed according to the method of Gao *et al*, 2006.

Fermentation

The slurries of cassava starch, dried chips and fresh roots (300 g dry solid l^{-1}) were initially liquefied by α-amylase (0.1% v/w) at $80^{O}C$ for 2 h. The sample was then prepared for a batch-typed Simultaneous Saccharification and Fermentation (SSF) in a 5-l fermenter with 2.5 l working volume. The samples were then adjusted to 150 g dry solid l^{-1} containing 5 g yeast extract, 40 g fish waste hydrolysate, 0.2 g $MgSO_4.7H_2O$, 0.005 g $MnSO_4.4H_2O$, 1.5 g sodium acetate, 1.5 g KH_2PO_4, and 1.5 g K_2HPO_4. The SSF was initiated by adding 0.5% (v/w) glucoamylase and seed culture (5% v/v). The agitation speed and temperature were regulated at 150 rpm and $40^{O}C$, respectively. The pH was controlled at 6.0 by adding 25% (w/v) NH_4OH.

Analytical assays

The fermentation samples were collected at different time intervals and used for the analysis. The weights of dry cells in the medium were determined after heating the samples at $105^{O}C$ for 24 h. The supernatant, collected after the centrifugation (at 10,000 rpm for 10 min) was used for determining lactic acid and residual sugar contents by a High Performance Liquid Chromatography (HPLC; Shimadzu, Japan), equipped with an Aminex HPX-87H column (Bio-Rad, USA) at $40^{O}C$, using 5 mM H_2SO_4 solution as an eluent with the flow rate of 0.6 ml min^{-1}.

Results and Discussion

Lactic acid can be produced by fermentation of sugars by lactic acid bacteria (LAB). Alternativetly, starch is used with the additional step of starch conversion to sugars by enzyme hydrolysis. Instead of using costly extracted cassava starch, less expensive cassava materials as dried chips and fresh roots, on dry basis containing mainly of starch, are considered to be a competitive feedstock. In this work, the lactic acid production from cassava starch (85% starch content, wet basis), chips (72.5% starch content, wet basis) and roots (26% starch content, wet basis) was performed with the substrate concentration of 150 g dry solid l^{-1}. Figure 1 exhibits changes in glucose and lactic acid concentrations during lactic acid production by *Lactobacillus rhamnosus* DM3 from different cassava materials. The glucose consumption and lactic acid production profiles were similar for all feedstocks evaluated. The glucose concentration was increased during the initial phase, reached the maximum at 6 h and started to decline afterwards. Yet, a complete sugar consumption of each substrate was achieved at different times, yielding different productivities (Table 1). Among all types of feedstock, the fermentation of cassava starch gave the highest productivity (4.69 g $l^{-1}h^{-1}$) whereas the productivity of fermenting fresh roots was lowest

(3.30 g $l^{-1}h^{-1}$). All significant parameters of fermentation performance still indicate the applicable uses of all cassava feedstock types for lactic acid production.

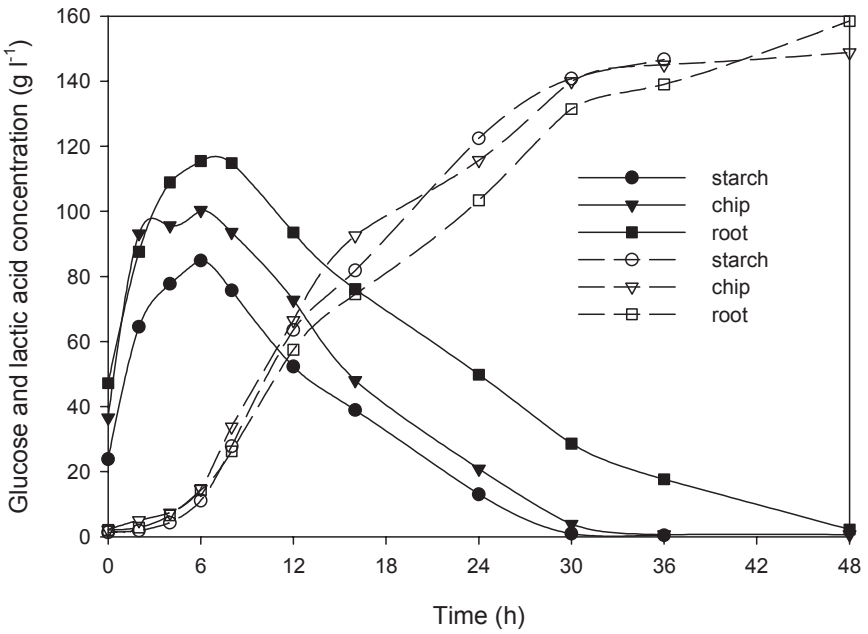

Figure 1 Profiles of glucose and lactic acid concentration (g l^{-1}) changes during lactic acid fermentation of various cassava materials including starch, dried chips and fresh roots by Homofermentative *Lactobacillus rhamnosus* DM3; close symbols and open symbols were glucose and lactic acid concentrations, respectively. (Fermentation condition: substrate concentration = 150 g l^{-1}, pH = 6, temperature = 40°C, agitation speed = 150 rpm).

Table 1 Parameters in lactic acid production of cassava hydrolysate from starch, dried chips and fresh roots* by Homofermentative *Lactobacillus rhamnosus* DM3.

Parameter	Starch	Dry chips	Fresh roots
Cell density at 620 nm.	17.83	24.46	24.35
Glucose residual (g l^{-1})	0.94	0.7	2.24
Starch consumption (g l^{-1})	150	150	148
Starch consumption (g)	375	375	369
Substrate utilization (%)	100	100	98.4
Total lactic acid concentration (g l^{-1})	140.8	145.1	158.5
Total lactic acid concentration (g)	359.0	370	404.2
$\%Yield_{P/S}$	95.7	98.7	109.5
Fermentation time (h)	30	36	48
Productivity (g $l^{-1}h^{-1}$)	4.69	4.03	3.30

*Cassava hydrolysate (150 g dry solid/l) was fermented at 40°C, pH by Simultaneous Saccharification and Fermentation (SSF) process with *Lactobacillus rhamnosus* DM3.

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