

Potential use of *Lactobacillus casei* TISTR 1500 for the bioconversion from palmyra sap and oil palm sap to lactic acid

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Received February 22, 2011 / Accepted May 21, 2011

Published online: September 15, 2011

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Abstract Lactic acid is a product that finds several applications in food, cosmetic, pharmaceutical and chemical industries. The main objective of this work is to evaluate potential use of the sap from palmyra (*Borassus flabellifer* Linn.) and oil palm (*Elaeis guineensis*) as substrate for lactic acid production by *Lactobacillus casei* TISTR 1500. The effects of acid hydrolysis, pH control and nutrient supplement of palmyra sap and oil palm sap on fermentation performance were investigated. It was found that lactic acid fermentation using palmyra sap was not significantly affected by either acid hydrolysis or pH control. The addition of MRS increased biomass and product yield. The final lactic acid concentration, dry cell weight and productivity were increased by increasing the total sugars of palmyra sap concentrations up to 134.0 g L⁻¹. The kinetic parameters for the palmyra sap at 134.0 g L⁻¹ total sugars were calculated to be of: specific growth rate (μ) 0.05 h⁻¹, the maximum productivity (R_M) 2.02 g lactic acid L⁻¹ h⁻¹, cellular yield coefficient ($Y_{X/S}$) 0.20 g cell g⁻¹ sugar, and lactic acid yield ($Y_{P/S}$) 0.78 g g⁻¹. When oil palm sap was used as carbon source for *L. casei* TISTR 1500, pH control did not significantly affect lactic acid production. The addition of MRS medium into oil palm sap improved the biomass and the product yield for which the lactic acid production in static flask at 37°C and pH 5.5 using 20 g L⁻¹ of total sugars was improved to be of 0.55 g L⁻¹ h⁻¹. Oil palm sap could be served as a good potential source of raw materials for efficient production of lactic acid by *L. casei* TISTR 1500.

Keywords: fermentation, lactic acid, *Lactobacillus casei* TISTR 1500, oil palm sap, palmyra sap

INTRODUCTION

Lactic acid (LA) is a versatile chemical, used as an acidant, flavour and preservative in food, and in pharmaceutical, leather and textile industries. It is also used for production of base chemicals, and for polymerization to produce biodegradable polylactic acid (PLA) (Hofvendahl and Hahn-Hägerdal, 2000). PLA could be a good substitute for synthetic plastic derived from petroleum feedstock (Kadam et al. 2006). Lactic acid has been produced commercially either by chemical synthesis or by microbial fermentation. Approximately, 90% of the total lactic acid produced worldwide has been obtained by bacterial fermentation, while the rest has synthesized by hydrolysis of lactronitrile. The chemical synthesis of lactic acid always results in race mixture of lactic acid, which is a major disadvantage. Fermentative production of lactic acid offers the advantages in both utilization of renewable carbohydrates and production of optically pure L- or D-lactic acid depending on the strain selected (Patil et al. 2006). *Lactobacillus casei* is an aciduric, rod-shaped, facultative heterofermentative lactic acid bacterium. *L. casei* is acid tolerant with an optimum pH of 5.5 and is relatively to product inhibition

by lactic acid (Bruno-Bárcena et al. 1999). The efficiency and economics of the ultimate lactic acid fermentation is however still a problem from many points of view and media compositions play a vital role in the improvement of such process. In recent years, research effort has focused on looking for new and effective nutritional source and new progressive fermentation techniques enabling the achievement of both high substrate conversion and high production yields (Bulut et al. 2004). A number of substrates have been used for biotechnological production of lactic acid, including glucose, sucrose, lactose, maltose, mannose, xylose, and galactose. The most pure product has been obtained when the pure sugar was fermented, resulting in the lower purification cost. However, there is economically unfavourable, because pure sugars are expensive and lactic acid is a relatively cheap product. To replace these refined and costly raw materials, the application of agricultural resources is promising to be used as an attractive resource because of their low prices (John et al. 2006; Tanaka et al. 2006). Using cheap raw materials as a fermentation substrate for lactic acid is an alternative way in reducing the cost of lactic acid production.

Palmyra palms (*Borassus flabellifer* Linn.) are abundant in the southern part of Thailand. They populate approximately 10 million plants. The most important product of palmyra palm is the sap. The total and reducing sugar in palmyra sap, which is rich in sucrose as dominant sugar, vary in the range of 10.36%-16.94% and 0.88%-3.56%, respectively (Naknean et al. 2010).

Oil palm (*Elaeis guineensis*) is widely planted for its edible oil in tropical countries such as Malaysia, Indonesia and Thailand. Palm oil is the most produced plant oil, with a worldwide production of 4.3 million tons in 2008. Oil palm sap was reported to contain large quantities of high glucose content sap. Glucose was found to be the dominant sugar in all parts, accounting for approximately 86.9%, 86.3% and 65.2% of the total free sugar contained in the inner, middle and outer parts, respectively (Kosugi et al. 2010).

In this study, we determined the physical and chemical composition of agricultural resources, palmyra sap, hydrolyzed palmyra sap and oil palm sap to evaluate their suitability as substrate for the production of lactic acid by *Lactobacillus casei* TISTR 1500, and to investigate the effect of acid hydrolysis of palmyra sap to obtain the sap that contains high fermentable reduced sugar for use as a substrate for bioconversion. The effects of pH control and nutrient supplementation of the palmyra sap, hydrolyzed palmyra sap and oil palm sap were also investigated for maximizing biomass and lactic acid production. The influence of pH on lactic acid production was studied by comparing fermentations between two conditions: with initial pH 5.5 that was allowed to vary, and with constant pH at 5.5 in stirred tank bioreactor.

MATERIALS AND METHODS

Microorganism and inoculums

Lactobacillus casei TISTR 1500, obtained from the Department of Biotechnology, Faculty of Agro-Industry, Rajamangala University of Technology Srivijaya, Thailand, was the microorganism used in the experiments. It was maintained at 4°C in plate culture on MRS (de Man, Rogosa, Sharpe) agar medium with the following composition (in g L⁻¹): proteose peptone, 10; beef extract, 10; yeast extract, 5; glucose, 20; polysorbate 80, 1; ammonium citrate, 2; sodium acetate 5; magnesium sulphate, 0.1; manganese sulphate, 0.05; dipotassium phosphate, 2; and agar, 15. The inoculum was prepared by transferring a loopful of cells to 250 mL conical flasks containing 50 mL sterile MRS broth (the same composition of MRS agar, but without agar). The flasks were incubated at 37°C for 24 hrs for seed culture. Ten millilitres of this culture was then transferred to a 250 mL Erlenmeyer flask containing 90 mL MRS broth, and incubated at the same conditions. Finally, the cells were harvested by centrifugation (8,000 rpm, 15 min) and directly resuspended in the fermentation medium to obtain a cell concentration of 1.0 g L⁻¹ at the beginning of the fermentation.

Raw material and characterization

Palmyra sap was collected from planters in Songkhla province, Thailand. The Palmyra sap contained pH, moisture content, total sugars (as sucrose) and total soluble solid of 4.30, 86.20%, 134.00 g L⁻¹, and 14.80°Brix, respectively. The Palmyra sap was hydrolyzed by adding 1 mL of 20% sulfuric acid in

100 mL of palmyra sap solution (Kadam et al. 2006). The acidified palmyra sap solution was heated in a boiling water bath for 20 min. The physical and chemical properties of hydrolyzed palmyra sap are listed as: pH 1.60, moisture content 87.88%, total sugars 181.53 g L⁻¹ (glucose 91.90 g L⁻¹ and fructose 89.63 g L⁻¹), and total soluble solid 17.60°Brix.

Oil palm sap was collected by using a laboratory-scale hydraulic press. The sap was centrifuged at 6,000 rpm for 15 min and the supernatant was stored at -20°C before use. The physical and chemical properties of oil palm sap are listed as: pH 7.49, moisture content 97.06%, total sugars 19.17 g L⁻¹ (glucose 16.58 g L⁻¹ and fructose 2.59 g L⁻¹), and total soluble solid 3.4°Brix. Average compositions of the samples are given in Table 1.

Table 1. Physical and chemical properties of palmyra sap, hydrolyzed palmyra sap and oil palm sap.

Parameters	Carbon source			Unit
	Palmyra sap	Hydrolyzed palmyra sap	Oil palm sap	
pH	4.30	1.60	7.49	-
Moisture	86.20	87.88	97.06	%
Total sugars	134.00	181.53	19.17	g L ⁻¹
glucose	-	91.90	16.58	g L ⁻¹
fructose	-	89.63	2.59	g L ⁻¹
sucrose	134.00	-	-	g L ⁻¹
Total soluble solid	14.80	17.60	3.40	°Brix

Lactic acid fermentation

L. casei TISTR 1500 was used for lactic acid fermentation experiments. The bacterium was pre-cultured on MRS medium. The palmyra sap and hydrolyzed palmyra sap were diluted to a final total sugars concentration of 20.0 g L⁻¹ while oil palm sap was used directly without dilution. The effect of nutrient supplement, pH control and carbon source (glucose, fructose, sucrose, palmyra sap, hydrolyzed palmyra sap and oil palm sap) on lactic acid production were evaluated. After formulation, 100 mL of the mediums were transferred to 250 mL Erlenmeyer flasks and sterilized at 121°C for 15 min. The flasks were statically incubated at 37°C for 72 hrs. Experiments for the evaluation of pH effect on lactic acid production were carried out in a 2 L stirred tank bioreactor with a working volume of 1 L. The culture temperature was 37°C. The pH was maintained at 5.5 with 2.0 N NaOH during fermentation. Samples were taken every 12 hrs and the dry cell weight, total sugars consumption and lactic acid production and productivity were compared to evaluate the process efficiency under different fermentation conditions. Reactor fermentation under each condition was carried out in triplicate, and data shown represent the mean values.

Analytical methods

Cell growth was measured by diluting the culture broth with distilled water to obtained optimum dilution. After mixing, the absorbance was measured by UV-spectrophotometer (UV-1601, Shimadzu, Japan) at 660 nm (Kurane et al. 1994). Dry cell weight was determined by centrifugation of culture broth (2 mL) at 8,000 rpm for 15 min. The cell sediments were dried for 24 hrs at 105°C and then weighed to constant weight after cooling in a desiccator (Dermlim et al. 1999). Lactic acid and acetic acid concentrations in supernatant were conducted by means of GC analysis. Gas chromatography (GC-14A, Shimadzu, Japan) was equipped with a BP-20 GC column (30 m x 0.53 mm) using flame ionization detector (Sura-Apinan et al. 2010). Residual sugar (sucrose, glucose and fructose) in the supernatant was determined by HPLC analysis, adapted from (Liu and Steinbüchel, 1997). Total sugars concentrations were analyzed by the Dubois method using phenol and sulphuric acid (Dubois et al. 1956).

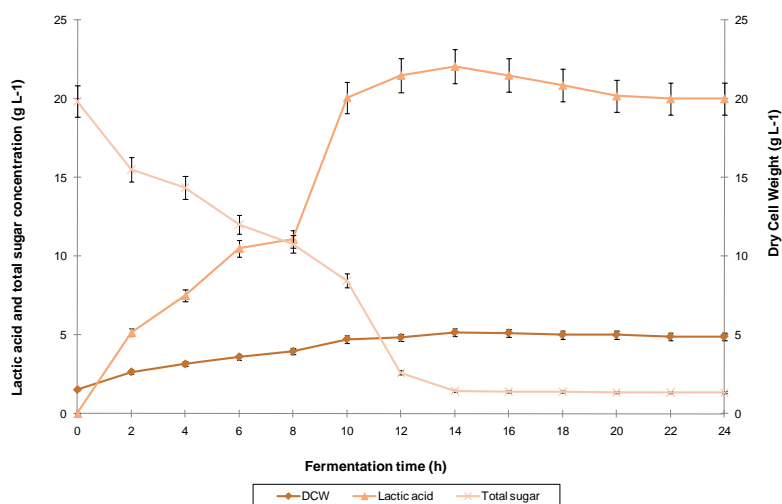


Fig. 1 Time course on lactic acid production in static flask cultivation with MRS Medium.

Fermentative parameters

The fermentation parameters were determined: the specific growth rate (μ , h^{-1}), defined as the ratio of logarithm of biomass concentration produced to elapsed time (h); cellular yield coefficient ($Y_{X/S}$, g g^{-1}), defined as the ratio of the total cell mass presented in the medium to sugar consumed; conversion yield of substrate to product ($Y_{P/S}$, g g^{-1}), defined as the ratio of lactic acid produced to sugar consumed; and maximum productivity (R_M , $\text{g L}^{-1} \text{h}^{-1}$), calculated as the ratio of lactic acid concentration to the fermentation time (Pirt, 1975).

RESULTS AND DISCUSSION

Lactic acid fermentation with a single carbon source (glucose, fructose, and sucrose)

Time course studies were conducted on growth and lactic acid production by *L. casei* TISTR 1500 in MRS medium (pH 5.5) using 20 g L^{-1} glucose as carbon source for 24 hrs at 37°C . The profile of growth (DCW), lactic acid production and total sugars utilization were shown in Figure 1. The bacterium grew rapidly within the first 14 hrs, correlating with the rapid decline of total sugars, which was due to the sugar being metabolized by the cells and the cell forming lactic acid. The maximum amount of lactic acid (22.06 g L^{-1}) was produced from 20 g L^{-1} glucose within 14 hrs of fermentation, with an increase in dry cell weight from 1.52 to 5.05 g L^{-1} . The kinetic parameters were as follows: specific growth rate (μ), 0.06 h^{-1} ; the product yield ($Y_{P/S}$), $1.20 \text{ g lactic acid g sugar}^{-1}$; cellular yield coefficient ($Y_{X/S}$), $0.20 \text{ g cell g sugar}^{-1}$; and the maximum productivity (R_M), $1.58 \text{ g lactic acid L}^{-1} \text{h}^{-1}$.

Table 2. Fermentation of *L. casei* TISTR 1500 using glucose, fructose and sucrose as the sole carbon source.

Carbon source	Dry Cell Weight (g L ⁻¹)	Final lactic acid (g L ⁻¹)	Residual sugar (g L ⁻¹)
Glucose	5.22 ± 0.02	22.06 ± 0.18	0.11 ± 0.04
Fructose	5.05 ± 0.01	21.15 ± 0.35	0.50 ± 0.08
Sucrose	4.78 ± 0.04	20.41 ± 0.21	0.10 ± 0.05

Note: Batch fermentations were performed on 250 mL static flasks with working volume of 100 mL at pH 5.5, 37°C for 14 hrs. Results were the average of data from triplicate experiments.

Typical kinetics of lactic acid formation by *Lactobacillus casei* TISTR 1500 during the fermentor cultivation with media containing glucose, fructose and sucrose as the carbon source were determined. When *L. casei* TISTR 1500 was cultivated on these three carbon sources, lactic acid formation patterns were similar. As shown in Table 2, the lactic acid production and the dry cell weight reached 20.41-22.06 g L⁻¹ and 4.78-5.22 g L⁻¹, respectively. Where the medium containing glucose was used as carbon source, *L. casei* TISTR 1500 could produce lactic acid more efficiently than fructose and sucrose. It is well known that sucrose is poorly metabolized by microorganisms compared to glucose (Bulut et al. 2004; Kadam et al. 2006; Alonso et al. 2010). Therefore, in order to hold the high ability of *L. casei* TISTR 1500 the hydrolysis of sucrose in palmyra sap to glucose was also conducted prior to fermentation.

Table 3. Effect of total sugars of palmyra sap on lactic acid production, dry cell weight and productivity.

Total sugars of palmyra sap (g L ⁻¹)	Residual sugar (g L ⁻¹)	Final lactic acid (g L ⁻¹)	Dry cell weight (g L ⁻¹)	Lactic acid yield (g g ⁻¹)	Productivity (g L ⁻¹ h ⁻¹)
10.0	0.58 ± 0.04	7.78 ± 0.97	1.30 ± 0.2	0.71 ± 0.06	0.56 ± 0.10
20.0	2.76 ± 0.50	12.00 ± 0.42	1.53 ± 0.1	0.72 ± 0.02	0.86 ± 0.04
40.0	16.46 ± 0.76	17.00 ± 0.28	2.75 ± 0.3	0.74 ± 0.02	1.21 ± 0.03
60.0	34.92 ± 0.12	18.95 ± 0.20	4.60 ± 0.2	0.76 ± 0.06	1.35 ± 0.02
134.0	97.00 ± 1.41	28.35 ± 0.22	8.51 ± 0.3	0.78 ± 0.02	2.02 ± 0.02

Note: Batch fermentations were performed on 250 mL static flasks with working volume of 100 mL with MRS medium at pH 5.5, 37°C for 14 hrs. Results were the average of data from triplicate experiments.

Lactic acid production from original palmyra sap

The influence of concentrations of original palmyra sap on lactic acid fermentation was determined through the culturing of *L. casei* TISTR 1500 on a static flask at 37°C, pH 5.5 and 14 hrs using 10.0-134.0 g L⁻¹ total sugars of palmyra sap. The obtained results (Table 3) showed that final lactic acid, dry cell weight and productivity increased with increasing total sugars of palmyra sap from 10.0 up to 134.0 g L⁻¹. The increase in product yields was 9.86% at 134.0 g L⁻¹ total sugars of palmyra sap in this study. However, the total sugars of palmyra sap consumption rate decrease with increasing total sugars of palmyra sap concentration, which resulted in the high total sugars concentration, would inhibit biomass and product formation. In addition, the most abundant sugar was sucrose, the high concentration of which made the viscosity of the liquid high (Bulut et al. 2004). Lactic acid yields, based on total sugars consumed were ranged between 0.71 g g⁻¹ and 0.78 g g⁻¹. The maximum dry cell weight was obtained up to 8.51 g L⁻¹ and the highest productivity of lactic acid was found to be 2.02 g L⁻¹ h⁻¹. All the maxima were obtained at 134.0 g L⁻¹ total sugars of palmyra sap. The optimum total sugars of palmyra sap for lactic acid fermentation by batch culture of *L. casei* TISTR 1500 seemed to be 134.0 g L⁻¹ total sugars of palmyra sap based on economic considerations of final lactic acid and productivity. However, total sugars of palmyra sap was not fully consumed in the presence of high total sugars of the palmyra sap and approximately 73% of the total sugars of the palmyra sap remained unused in the fermentation medium. The kinetic parameters of the palmyra sap of 134.0 g L⁻¹ total sugars revealed that the maximum productivity (R_M) was 2.02 g lactic acid L⁻¹ h⁻¹ and the lactic acid yield ($Y_{P/S}$) was 0.78 g g⁻¹.

The profile of growth (dry cell weight), pH, lactic acid production and total sugars utilization were shown in Figure 2. The total sugars of palmyra sap concentrations of 10.0, 20.0, 40.0, 60.0, and 134.0 g L⁻¹ provided rapid growth rate (maximum dry cell weight) of 1.30, 1.53, 2.75, 4.60 and 8.51 g L⁻¹, respectively. Lactic acid from *L. casei* TISTR 1500 increased as total sugars concentrations increased up to 134.0 g L⁻¹. Therefore, the carbon source concentration affected the efficiency of substrate conversion to lactic acid. The high carbon source concentration resulted in high lactic acid concentration. In this study, acetic acid was the by-products in the fermentation broth (0-1.43 g L⁻¹) (Figure 2d). Ethanol and formic acid were not detected. Acetic acid could be occurred by both post-pyruvate and pre-pyruvate (Hofvendahl and Hähn-Hagerdal, 2000). Therefore, not only heterofermentation occurred but also mixed acid fermentation occurred. The hetero- or mixed acid fermentation routes give not only lactic acid, but formic acid and acetic acid as byproducts (Vickroy, 1985).

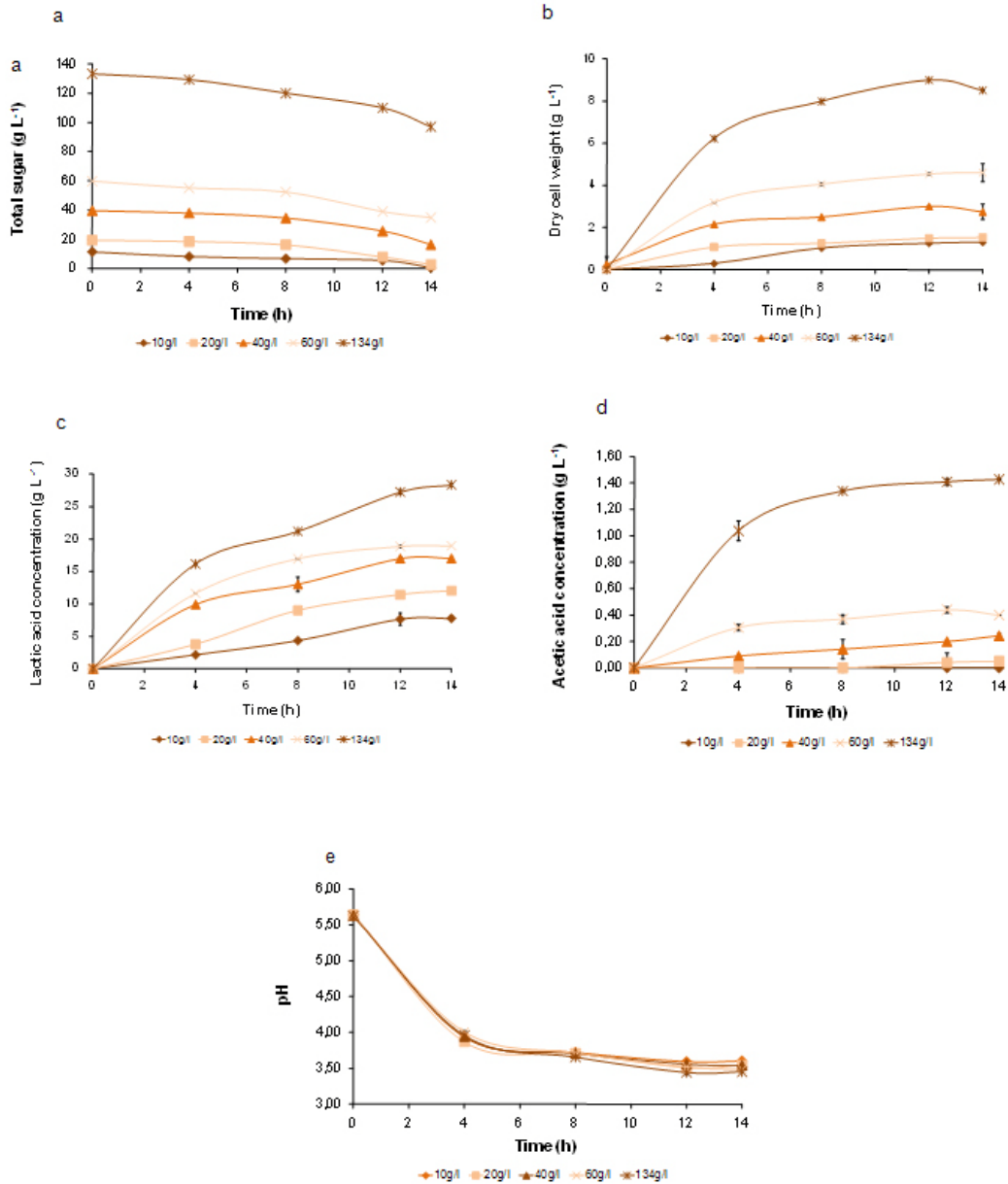


Fig. 2 Kinetic profiles of total sugars consumption (a), dry cell weight (b), lactic acid concentration (c), acetic concentration (d) and pH (e) over time of fermentation for lactic acid production by *L. casei* TISTR 1500 with: 10 g L⁻¹, 20 g L⁻¹, 40 g L⁻¹, 60 g L⁻¹, 134 g L⁻¹ total sugars of palmyra sap.

Although total sugars concentration at 134.0 g L⁻¹ gave the highest biomass and lactic acid production, the total sugars of palmyra sap at 20 g L⁻¹ was selected as the total sugars concentration to improve the economic carbon source from palmyra sap containing the same sugar concentration as in MRS media that contains 20 g L⁻¹ glucosein lactic acid production.

Effect of medium supplementation on lactic acid production

Experiments were initially carried out to investigate the influence of fermentation performance with and without MRS medium addition to the palmyra sap, hydrolyzed palmyra sap and oil palm sap for lactic acid production in static flask at 37°C and pH 5.5 using 20 g L⁻¹ of total sugars. The results, shown in

Figure 3, clearly demonstrated that *L. casei* TISTR 1500 slightly grew in the without-addition MRS medium of the palmyra sap, hydrolyzed palmyra sap and oil palm sap. This might be due to the effects of some nutrient source presenting in these saps but there were insufficient nutrients for growth of bacteria. Products from fermentation were increased against time and were relatively constant at 42 hrs. The two highest main product concentrations detected were lactic acid and acetic acid. Palmyra sap, hydrolyzed palmyra sap and oil palm sap contained sufficient sugar content to be used as carbon source for lactic acid production. However, MRS media, which contains yeast extract, peptone and meat extract, was supplemented to these saps to support growth as the saps did not contain significant amount of nitrogen and minerals.

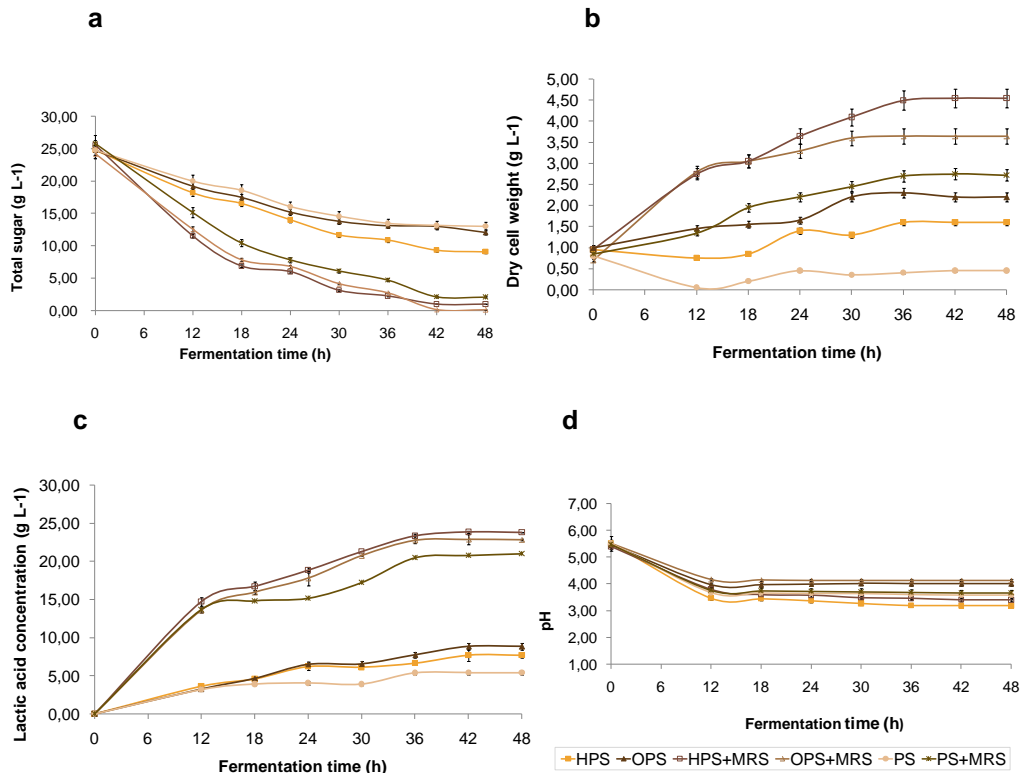


Fig. 3 Kinetic profiles of total sugars consumption (a), dry cell weight (b), lactic acid production (c) and pH (d) over time of fermentation for lactic acid production by *L. casei* TISTR 1500 with: PS, HPS, OPS, PS+MRS, HPS+MRS, OPS+MRS.

In Figure 3 a strong *L. casei* TISTR 1500 growth was observed. Results clearly showed that the palmyra sap, hydrolyzed palmyra sap and oil palm sap with addition MRS medium improved the fermentation by *L. casei* TISTR 1500 compared to the without-addition MRS medium. Nancib et al. (2005) using date juice and *Lactobacillus casei* subsp. *rhamnosus* NRRL-B445, reported that supplementation with yeast extract increased lactic acid production compared with unsupplemented date juice because *Lactobacilli* have complex nutrient requirements. Therefore, to achieve optimal cultivation conditions, the fermentation medium should contain minerals, B-vitamins, amino acids, fatty acids, purines and pyrimidines for bacteria growth and biological activity. Hofvendahl and Hahn-Hägerdal (2000) compared several studies concerning lactic acid production in fermentation media supplemented with different kinds of nutrients and reported on the positive aspect that addition of MRS broth components promotes a better fermentation performance when compared with addition of yeast extract. This could be explained by considering that yeast extract is also present in the MRS medium composition together with other nutrients such as meat extract, peptone and some salts.

Figure 3a showed sugar consumption in with and without addition MRS medium to the palmyra sap, hydrolyzed palmyra sap and oil palm sap. The consumption of total sugars (91.78-99.38%) in the with-

addition MRS medium of the palmyra sap, hydrolyzed palmyra sap and oil palm sap was higher than that in the without-addition MRS medium (46.93-62.31%). The total sugars uptake in the with-addition MRS medium to these saps continued until the end of fermentation (42 hrs). Cell growth was favoured in the palmyra sap, hydrolyzed palmyra sap and oil palm sap with the addition MRS medium (Figure 3b). The bacterial grew rapidly within 36 hrs and ceased after that. This was correlated to the rapid decline of pH due to that the sugar was metabolized by cells to form acidic metabolites. This caused growth inhibition and lactic acid production.

Table 4. Kinetic parameters of lactic acid production by *L. casei* from different carbon sources.

Carbon source	Residual sugar (g L ⁻¹)	Lactic acid (g L ⁻¹)	Yield (g g ⁻¹)	Maximum DCW (g L ⁻¹)	Productivity (42 hrs) (g L ⁻¹ h ⁻¹)
PS	13.00 ± 0.42	5.41 ± 0.20	0.46 ± 0.05	0.45 ± 0.03	0.13 ± 0.007
HPS	9.10 ± 0.28	7.71 ± 0.18	0.49 ± 0.02	1.60 ± 0.02	0.18 ± 0.006
OPS	12.10 ± 0.28	8.85 ± 0.12	0.70 ± 0.02	2.20 ± 0.05	0.21 ± 0.004
PS+MRS	2.08 ± 0.11	20.81 ± 0.21	0.88 ± 0.01	2.72 ± 0.04	0.50 ± 0.007
HPS+MRS	0.98 ± 0.03	23.87 ± 0.23	0.97 ± 0.06	4.55 ± 0.03	0.57 ± 0.008
OPS+MRS	0.13 ± 0.03	22.90 ± 0.15	0.95 ± 0.01	3.65 ± 0.04	0.55 ± 0.005

Note: Batch fermentations were performed on 250 mL static flask with working volume of 100 mL at pH 5.5, 37°C for 42 hrs. Results were the average of data from triplicate experiments. PS: palmyra sap; HPS: hydrolyzed palm sap; OPS: oil palm sap; MRS: deMan Rogosa and Sharpe.

It is worth emphasizing that all these assays were performed without pH control which clearly affected the fermentation performance. As shown in Figure 3d, at the beginning of the process (12 hrs), pH decreased from 5.53 to 3.48 in all fermentation media as a consequence of lactic acid production by the microorganism. This affected the microorganism metabolism that acted better in a pH range between 5.0 and 7.0 (Lasekan et al. 2007). Moreover, pH 5.5 has been used for lactic acid production using *L. helveticus* (Ghaly et al. 2004). Hydrogen ion concentration of a medium had the maximum influence on microbial growth. The pH has affected at least two aspects of microbial cells, *i.e.* functioning of its enzymes and the transport of nutrients into the cell. It has limited the synthesis of metabolic enzymes responsible for the synthesis of new protoplasm. In addition, the pH values still affect the RNA and protein synthesis. When microorganisms were grown on either side of their optimum pH range, there may be an increasing lag phase.

The kinetic parameters of batch cultivation in static flask were given in Table 4. Kinetics values obtained from cultivation with MRS medium in the palmyra sap, hydrolyzed palmyra sap and oil palm sap were higher than those from the palmyra sap, hydrolyzed palmyra sap and oil palm sap without the MRS medium. Lactic acid yields, based on total sugars consumed, were obtained in the range of 46.0-97.0%. Moreover, the maximum lactic acid productivity (0.57 g L⁻¹ h⁻¹) and the dry cell weight (4.55 g L⁻¹) were found in the MRS-contained hydrolyzed palmyra sap.

Table 5. Lactic acid production for various sources of carbon, acid hydrolysis and MRS medium during 42 hrs of flask cultivation (initial total sugars concentration: 20 g L⁻¹).

Carbon source	Enhancement of product yield (%)	
	by acid hydrolysis	by MRS medium
Palmyra sap	6.52	91.30
Hydrolyzed palm sap	-	97.96
Oil palm sap	-	35.71

Results were shown in Table 5. The addition of MRS medium enhanced the product yield of palmyra palm (91.30%), hydrolyzed palmyra sap (97.96%) and oil palm sap (35.71%). There was a slight significant increase in the amount of product yield (6.52%) when palmyra sap with MRS medium was hydrolyzed. The reason was that acid hydrolysis using sulfuric acid had caused releasing of some toxic compounds or inhibitors (Laopaiboon et al. 2010). Thus, palmyra sap with MRS medium could well serve as a carbon source for *L. casei* TISTR 1500 without acid hydrolysis.

Effect of pH control on fermentation

Level of pH is one of the most important environment parameters affecting cell growth and product formation. In general, effects of pH on cell growth and product accumulation vary with different microorganisms, medium compositions, and operational conditions. Some literatures (Hofvendahl and Hahn-Hagerdal, 2000; Chang et al. 2001; Idris and Suzana, 2006) dealing with conditions of lactobacillus reported the optimal pH for cell growth and lactic acid production. To date, no reports have been found about the effects of pH control on cell growth and lactic acid production in a lab-scale fermentor with palmyra sap, hydrolyzed palmyra sap and oil palm sap as substrates. Lactic acid-producing bacteria (LAB) are constantly confronted with acidified environments, making acid stress part of the life cycle of LAB due to their ability to ferment sugars into lactate. Knowledge on metabolic process stresses response caused by low pH in certain strains of development of many biotechnology products. The purpose of this experiment was to evaluate the inhibitory effect on growth and lactic acid production by *L. casei* TISTR 1500 exposed to conditions of stress caused by acidification of the medium. In this work, all fermentation cases started in 2 L fermentor containing 1 L optimal medium, incubation temperature 37°C, with and without control of pH (5.5). The results were displayed in Figure 4.

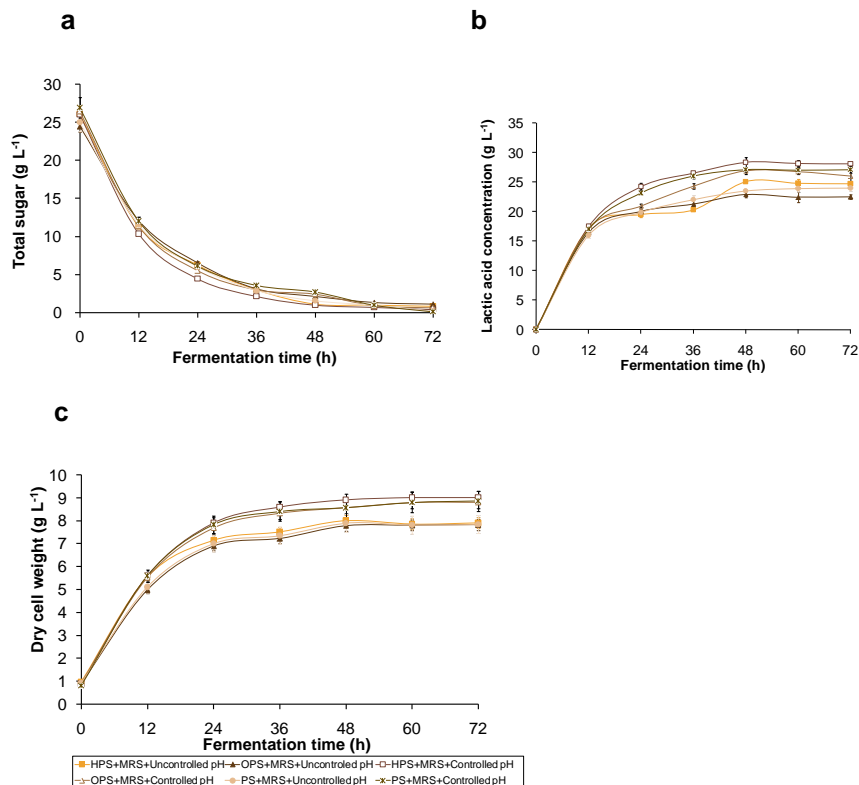


Fig. 4 Effect of pH control on total sugars consumption (A), lactic acid production (B) and dry cell weight of *L. casei* TISTR 1500 (C) in media: palmyra sap with MRS components without pH control; with pH control; hydrolyzed palmyra sap with MRS components without pH control; with pH control; oil palm sap with MRS components without pH control; with pH control.

First, it is important to note that microorganisms were able to grow and produced lactic acid in both culture media tested (with, and without pH control). There was a similar growth pattern, reaching a stationary phase after 48 hrs of fermentation. During the initial 12 hrs, a similar performance was observed in fermentations with and without pH control. However, the consumption of total sugars, lactic acid production and cell growth were influenced by the fermentation pH (Figure 4).

According to some authors (Kashket, 1987; Hofvendahl and Hahn-Hägerdal, 2000), weak acid, e.g., lactic acid, inhibit bacterial growth because as the external pH declines, the acid is protonized as soon as it is exported out of the bacteria. Uncharged, it diffuses back into the cell and dissociates due to higher intracellular pH. The cell then has to use ATP to pump out protons and energy eventually is depleted, causing growth stop and the bacteria die.

Lactic acid production in pH-controlled palmyra sap, hydrolyzed palmyra sap and oil palm sap supplemented with MRS components were of 27.12 g L⁻¹, 28.34 g L⁻¹ and 26.49 g L⁻¹, respectively. While in the medium without pH control only 23.50 g L⁻¹, 25.08 g L⁻¹ and 22.90 g L⁻¹ were obtained (Figure 4b). These values represented approximately increasing in lactic acid concentration of 15%, 13% and 16%, respectively, when the pH of the MRS-supplemented palmyra sap, hydrolyzed palmyra sap and oil palm sap was controlled. Alonso et al. (2010) using *Lactobacillus casei* ATCC 393 and yoghurt whey, reported this same behavior.

Microorganism was able to grow and produce lactic acid with highest efficiency in the MRS-supplemented hydrolyzed palmyra sap, which could have favoured the bioconversion process since the higher the cell concentration the larger the amount of substrate can be consumed and converted into product (Figure 4c).

According to Idris and Suzana (2006) lactic acid production depends on microbial growth, thus an increase in microbial growth promotes an increase in the lactic acid production. Mussatto et al. (2008) found that after 60 hrs fermentation lactic acid production by *L. delbrueckii* UFV H2B20 in brewer's grain cellulosic hydrolysate supplemented with MRS components, and pH controlled value at 6.0, $Y_{P/S}$ and R_M values of 0.99 g g⁻¹ and 0.59 g L⁻¹ h⁻¹, respectively, were obtained.

Table 6 summarized the fermentative parameters obtained in the fermentation runs with and without pH control. It was found that lactic acid yield and productivity were higher than those in the fermentation runs with pH control. The reason could be because the pH inhibition and the low cell growth provoked the inability of the strain in using the remaining sugars. As the fermentation continued, the rate slowed down because of the accumulation of lactic acid so pH values fell causing metabolic inhibition. This indicated the importance of pH control. The result was obtained similarly by Tango and Ghaly (1999). They reported that productions of 10 g L⁻¹ of lactic acid from fermentation of cheese whey without pH control using *Lactobacillus helveticus*. The pH inhibition and the final lactic acid concentration were suggested to have a strong inhibitory effect.

Table 6. Fermentation parameters of lactic acid production by *L. casei* TISTR 1500 in different fermentation media.

	PS+MRS		HPS+MRS		OPS+MRS	
	Without pH control	With pH control	Without pH control	With pH control	Without pH control	With pH control
Lactic acid (g L ⁻¹)	23.50 ± 0.250	27.12 ± 0.360	25.08 ± 0.120	28.34 ± 0.350	22.90 ± 0.240	26.89 ± 0.400
Acetic acid (g L ⁻¹)	0.20 ± 0.030	0.22 ± 0.050	0.22 ± 0.040	0.23 ± 0.110	0.25 ± 0.120	0.26 ± 0.080
$Y_{P/S}$ (g g ⁻¹)	0.95 ± 0.061	1.01 ± 0.022	0.99 ± 0.004	1.11 ± 0.012	0.98 ± 0.049	1.04 ± 0.001
$Y_{X/S}$ (g g ⁻¹)	0.28 ± 0.015	0.30 ± 0.008	0.27 ± 0.011	0.32 ± 0.003	0.30 ± 0.005	0.31 ± 0.020
R_M (g L ⁻¹ h ⁻¹)	0.49 ± 0.007	0.56 ± 0.011	0.52 ± 0.004	0.59 ± 0.010	0.48 ± 0.007	0.56 ± 0.012

Note: Batch fermentations were performed on a 2.0 L stirred tank bioreactor with 1 L working volume at pH 5.5, 37°C for 48 hrs. Results were the average of data from triplicate experiments. PS: palmyra sap; HPS: hydrolyzed palm sap; OPS: oil palm sap; deMan Rogosa and Sharpe.

It was found that the kinetic parameters of pH controlled of the MRS-supplemented hydrolyzed palmyra sap were highest with the following results: conversion yield of substrate to product ($Y_{P/S}$) 1.11 g lactic acid g^{-1} total sugars, cellular yield coefficient ($Y_{X/S}$) 0.32 cells g^{-1} total sugars, and the maximum productivity (R_M) 0.59 g lactic acid $L^{-1} h^{-1}$ in this study. In the culture, both for the uncontrolled and controlled pH, not only lactic acid but also acetic acid (0.20-0.26 $g L^{-1}$) were the main products in the fermentation broth. These results indicated that glucose, the main sugar in the hydrolyzed palmyra sap and oil palm sap, was metabolized to lactic acid via heterolactic fermentation pathway or mixed acid fermentation.

As tabulated in Table 7, with pH control of palmyra sap, hydrolyzed palmyra palm and oil palm sap, product yield were of 6.32%, 12.12% and 6.12%, respectively. This indicated that the effect of pH control of palmyra sap, hydrolyzed palmyra palm and oil palm sap rendered only a slight significant increase in lactic acid production. Ha et al. (2003) studied on lactic acid production by *Lactobacillus casei* KH-1. In their study, the product yield increased 13.67% with pH controlled batch cultures using MRS medium. Moreover, Alonso et al. (2010) also found that the pH control showed 5.56% and 26.76% improvement in lactic acid yield on substrate with and without yeast supplementation by *Lactobacillus casei* ATCC 393. Therefore, pH control from palmyra sap, hydrolyzed palmyra palm and oil palm sap might not be required in this study. Cost-effectiveness of controlled versus uncontrolled pH for lactic acid production should be further studied.

Table 7. Lactic acid production with pH control for various sources of carbon during 42 hrs of bioreactor cultivation (initial total sugars concentration: 20 $g L^{-1}$).

Carbon source	Enhancement of product yield by pH control (%)
Palmyra sap	6.32
Hydrolyzed palm sap	12.12
Oil palm sap	6.12

With increasing interests in producing biotechnological products from low-cost and renewable biomass, production of lactic acid from various raw agricultural materials has gained considerable attentions recently. Many microorganisms, including lactic acid bacteria (LAB), have been investigated for production of lactic acid. Some examples of microbial lactic acid production from agricultural resources by LAB were compared with this work. Relatively low productivity of lactic acid was obtained when oil palm sap by *Lactobacillus lactis* ATCC 19435 (Kosugi et al. 2010) used for lactic acid production. However, higher productivity of lactic acid were reported using hydrolyzed cane sugar by *Lactobacillus delbrueckii* (Kadam et al. 2006), wheat bran by *Lactobacillus rhamnosus* (Li et al. 2010), molasses by *Lactobacillus delbrueckii* NCIMB 8130 (Kotzamanidis et al. 2002), and cashew apple juice by *Lactobacillus casei* B-442 (Silveira et al. 2010). In the present study, productivity of palmyra sap (2.02 $g L^{-1} h^{-1}$ at 134.00 $g L^{-1}$ total sugars) less than many of those reported since it contained high sucrose content raw material, which can only be slowly metabolized by lactic acid bacteria. In contrast, high productivity of lactic acid could be obtained by using oil palm sap (0.55 $g L^{-1} h^{-1}$ at 20.00 $g L^{-1}$ total sugars) compared to palmyra sap. Hence, oil palm sap is potentially more feasible and more efficient in lactic acid production by *L. casei* TISTR 1500.

CONCLUDING REMARKS

This study has shown the potential use of *L. casei* TISTR 1500 for bioconversion of agricultural resources including palmyra sap and oil palm sap to reduce the manufacturing cost of lactic acid production. Lactic acid fermentation using palmyra sap was not significantly affected by acid hydrolysis and also by pH control. The final lactic acid concentration, dry cell weight and productivity increased with the increases of total sugars of palmyra sap concentrations up to 134.0 $g L^{-1}$. Improved biomass and lactic acid production of *L. casei* TISTR 1500 cultured in palmyra sap have been achieved by MRS medium. The best bioconversion performance was attained under these conditions: specific growth rate (μ), maximum productivity (R_M), cellular yield coefficient ($Y_{X/S}$) and lactic acid yield ($Y_{P/S}$) values of

0.05 h⁻¹, 2.02 g lactic acid L⁻¹ h⁻¹, 0.20 g cell g⁻¹ sugar, and 0.78 g g⁻¹, respectively. Lactic acid production by *L. casei* TISTR 1500 in oil palm sap was influenced by MRS supplementation. However, there were no significant increases in the amounts of biomass and product yield when pH of the oil palm sap with MRS medium was controlled. The highest values of production yield and maximum productivity obtained from static condition at 37°C, pH 5.5 and 20 g L⁻¹ of total sugars were 0.95 g g⁻¹ and 0.55 g L⁻¹ h⁻¹, respectively. Oil palm sap was proven to be a great potential raw material for lactic acid production by *L. casei* TISTR 1500.

Financial support: This work was financially supported by grants from graduate school Prince of Songkla University, Thailand.

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How to reference this article:

CHOOKLIN, S.; KAEWSRICHAN, L. and KAEWSICHAN, J. (2011). Potential use of *Lactobacillus casei* TISTR 1500 for the bioconversion from palmyra sap and oil palm sap to lactic acid. *Electronic Journal of Biotechnology*, vol. 14, no. 5. <http://dx.doi.org/10.2225/vol14-issue5-fulltext-11>