

*Original Article*

# Lactic acid production from alkaline hydrothermal reaction of cassava starch

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**Abstract**

Carbohydrate content in biomass is a suitable starting material to serve as a renewable feedstock for chemical products such as bio-degradable plastic and polylactic acid. Cassava starch is a common substrate for lactic acid production from fermentations, but rarely seen as a starting material in lactic acid production by a chemical method. This study shows that cassava starch is also a suitable starting material for a chemical process to produce lactic acid by the alkaline hydrothermal method assisted by microwave radiation. The effects of base catalysts, reaction temperature, catalyst concentration, and reaction time were investigated. In comparison with the lactic acid productivities obtained from cassava starch fermentations, the hydrothermal reaction method developed in this study gave higher volumetric productivities but lower lactic acid mass to cassava starch mass ratios. The advantages of the hydrothermal method also include the short operating time and low cost of the catalyst.

**Keywords:** cassava starch, lactic acid, alkaline hydrothermal reaction, microwave radiation, biomass

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**1. Introduction**

Cassava starch is a major renewable agricultural resource in Thailand. Starch serves as a carbohydrate energy storage substance in higher order plants. Starch is a versatile natural polysaccharide that consists of a large number of glucose units. Starch consists of two polymers: amylose and amylopectin in the ratio of 1 to 3. Amylose is a linear polymer of  $\alpha$ -D-glucopyranosyl units joined by  $\alpha(1\rightarrow4)$  glycosidic bonds. The degree of polymerization (DP) of amylose is around 250–1000 D-glucose units. Amylopectin is highly branched and is also composed of  $\alpha$ -D-glucopyranosyl monomers. Its linear chains are also joined by  $\alpha(1\rightarrow4)$  glycosidic bonds. Amylopectin is much larger than amylose, with a molecular weight on the order of  $10^6$  to  $10^8$  g/mol, corresponding to a DP of around 5000 to 50,000 D-glucose units (Wrolstad, 2012). Cassava roots are a major source of starch, and they are abundantly available (Adetunji, Isadare,

Akinluwade, &amp; Adewoye, 2015).

Cassava starch is normally processed into starch, chips, pellets, and as food and animal feed. In Thailand, the industrial production of cassava has been extended from pellets and chips to value-added products, such as starch and its derivatives, including modified starch, sweetener, tapioca pearls, sugar alcohols, alcohols, and organic acids (Adetunji *et al.*, 2015; Piyachomkwan & Tanticharoen, 2011).

Starch is used as a feedstock for chemical production, such as lactic acid by fermentation (Afolabi, Sosu-Mobee, & Abdulkareem, 2012; Altaf, Naveena, Venkateshwar, Kumar, & Reddy, 2006; Chookietwattana, 2014; Linko & Javanainen, 1996; Roble, Ogbonna, & Tanaka, 2003; Sridevi, Padmaja, Sahitya, Vardhan, & Rao, 2015; Xiaodong, Xuan, & Rakshit, 1997; Yin *et al.*, 1997; Yuwono, Hadi, Fitriani, & Kokugan, 2011). Lactic acid is a common building block for the polymer industry to produce biodegradable polymer and polylactic acid (PLA). Due to current global environmental problems, the global demand for PLA is increasing, and the production of lactic acid with a low cost starting material and a simple technique is necessary and remains challenging (Grand View Research, 2014). Any carbohydrate biomass can be used as a starting material in

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producing lactic acid (Castillo Martinez *et al.*, 2013). Lactic acid is commonly produced by fermentation methods. An L-lactic acid yield of 85% was produced from the 3-day fermentation of corn starch by *Rhizopus oryzae* (Yin *et al.*, 1997). The fermentations of different types of starch by *Lactobacillus* provided lactic acid yields from 19% to 98% after incubation from 24 h to 5 days (Altaf *et al.*, 2006; Chookietwattana, 2014; Linko & Javanainen, 1996; Sridevi *et al.*, 2015). According to the results from previous studies, lactic acid production by fermentation processes typically takes 24 h or longer. Additionally, fermentation methods require bacterial media along with protein supplements which are relatively expensive. Fermentations also require complicated bioreactor equipment and set-ups in addition to long fermentation times. Thus, lactic acid production through a chemical technique may be an alternative method that requires a much shorter time and lower production cost.

Chemical methods have advantages, such as applicability for industrial scale production, short reaction time, and low production cost (Zhang, Jin, Hu, & Huo, 2011). The alkaline hydrothermal technique, which requires a base catalyst and high temperature and pressure conditions, is a promising method for the production of lactic acid (Bicker, Endres, Ott, & Vogel, 2005; Cantero, Vaquerizo, Martinez, Bermejo, & Cocero, 2015).

Microwave radiation has been regarded as a new environmentally friendly heat source technology for organic synthesis (Joshi, 2013). Microwave radiation heating reduces reaction times and increases reaction rates. Thus, heating by microwave radiation is suitable for the alkaline hydrothermal reaction (Ameta, Punjabi, Ameta, & Ameta, 2014; Sharifi, Mojtahedi, & Saidi, 1999).

The production of lactic acid from the alkaline hydrothermal reaction of glucose has been studied. A 28% yield of lactic acid was obtained from glucose under hydrothermal reaction conditions with 0.32 M Ca(OH)<sub>2</sub> at 300 °C for 1 min (Jin & Enomoto, 2007). In addition, an investigation into the performance of NaOH and Ca(OH)<sub>2</sub> as alkaline catalysts for lactic acid production from potato starch, cellulose, and glucose was conducted. Lactic acid yields of 20%, 19.2%, and 18.7% were obtained from the hydrothermal reaction of 0.32 M Ca(OH)<sub>2</sub> with glucose, cellulose, and potato starch, respectively at 300 °C after 1 min (Yan, Jin, Tohji, Kishita, & Enomoto, 2010). The alkaline hydrothermal reaction of biomass, assisted by microwave radiation, is proposed to be an efficient technique in producing lactic acid (Ameta *et al.*, 2014; Mendes *et al.*, 2012).

For tropical countries including Thailand, where starch can be abundantly produced, starch is a more suitable feedstock than other types of biomass because its particle components are finer and may not require much biomass pretreatment. In addition, starch particles have more surface area, which is an important factor for heterogeneous reactions such as the hydrothermal reaction.

This research focused on determining whether cassava starch would also be a suitable feedstock for chemical conversion into lactic acid. Cassava starch, which is commonly used as a biomass feedstock for lactic acid production by fermentation, was utilized as the starting material for a hydrothermal process to produce lactic acid in this study. A set of experimental designs was performed in order to observe the effects of reaction parameters on lactic

acid production yield. The effects of catalyst types, reaction temperatures, reaction times, and catalyst concentrations were investigated on lactic acid production from the microwave radiated hydrothermal reaction.

## 2. Materials and Methods

### 2.1 Materials and reagents

Cassava starch was donated from Siam Modified Starch Industry Co. Ltd. D-glucose was purchased from Fluka Company. Barium hydroxide, calcium hydroxide, and sodium hydroxide were purchased from Ajax Finechem Pty Ltd, Australia. Magnesium hydroxide, potassium hydroxide, acetic acid (100%), formic acid (98-100%), 1,3-propanediol, and sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, 97%) were purchased from Merck, Germany. Lactic acid was purchased from Combi-Blocks Inc., USA.

### 2.2 Alkaline hydrothermal reaction

All experiments of hydrothermal conversion of cassava starch catalyzed by alkaline bases were carried out in a CEM MARS 6 microwave reactor. Cassava starch was mixed with a base solution at the desired concentrations. Reaction temperature, reaction time, pressure, and power were controlled by classical modes in the CEM MARS 6. The crude products obtained from the alkaline hydrothermal reactions with microwave radiation were filtered and kept for analytical testing. All experiments were performed at least in triplicate, and the average values were reported.

#### 2.1.1 Investigations on catalyst types

A set of experiments starting with a screening of catalyst types was conducted to investigate lactic acid production from the alkaline hydrothermal method. The reactions were carried out with magnesium hydroxide (Mg(OH)<sub>2</sub>), barium hydroxide (Ba(OH)<sub>2</sub>), calcium hydroxide (Ca(OH)<sub>2</sub>), sodium hydroxide (NaOH), and potassium hydroxide (KOH) as catalysts. It should be noted that Ba(OH)<sub>2</sub> is a toxic chemical. Precautions during use and disposal of Ba(OH)<sub>2</sub> should be taken (Young, 2006). All experiments were carried out in the closed system of the CEM Mars 6 microwave reactor with a 20 mL Easy Prep vessel. Cassava starch (200 mg) was mixed with various base solutions (20 mL) at 0.05 M. The alkaline hydrothermal reactions were carried out at 240 °C for 30 min under the maximum pressure of 600 PSI and power of 1500 W.

#### 2.1.2 Investigation on the effect of reaction temperature

A set of experiments was performed at various reaction temperatures (180, 200, 220, and 240 °C) for 30 min in a CEM Mars 6 microwave reactor. Cassava starch (200 mg) was mixed with 20 mL of 0.05 M Ba(OH)<sub>2</sub>. A reaction temperature of 240 °C was set as the highest temperature in this study because it was found that the yield of lactic acid from cellulose did not increase at a temperature higher than 240 °C (Wang, Liu, & Dong, 2013). However, a temperature of 180 °C was found to be the most suitable reaction

temperature for lactic acid production from glucose with potassium hydroxide under solvent-free conditions assisted by microwave heating (Epane, Laguerre, Wadouachi, & Marek, 2010).

### 2.1.3 Investigation on the effect of catalyst concentration on lactic acid yield

In each experiment, cassava starch (200 mg) was mixed with various Ba(OH)<sub>2</sub> concentrations (0.05, 0.1, 0.15, and 0.2 M). The reaction was performed at 240 °C for 30 min.

### 2.1.4 Reaction time effect on lactic acid

The last set of experiments was performed to see the effect of reaction time on lactic acid yield. A mixture of cassava starch (200 mg) with 20 mL of 0.15 M Ba(OH)<sub>2</sub> was subjected to the hydrothermal reaction at 240 °C for 30 min and 60 min under a CEM Mars 6 microwave reactor. In order to increase the volumetric productivity (Sirisansaneeyakul, 2013), another two sets of experiments were conducted at the reaction times of 60 min but at higher concentrations of starting material. The experiments were carried out with 1000 mg or 2000 mg of cassava starch in 50 mL of 0.15 M Ba(OH)<sub>2</sub> under the hydrothermal reaction conditions at 240 °C for 60 min using the CEM Mars 6 microwave reactor.

### 2.1.5 Product analysis

Crude products were analyzed by high performance liquid chromatography (HPLC). The concentrations of lactic acid, formic acid, and acetic acid were calculated based on a standard curve constructed from the corresponding standard compound. Each crude sample was diluted and adjusted to pH 3 by 0.1 M H<sub>2</sub>SO<sub>4</sub> and filtered with 0.22 µm filter paper. The samples were analyzed by HPLC (Agilent 1260 series), equipped with VertiSep™ OA column (7.8 x 300 mm, flow rate 0.6 mL/min) using isocratic 0.01 N H<sub>2</sub>SO<sub>4</sub> as the mobile phase (Esposito & Antonietti, 2013). 1,3-Propanediol was used as an internal standard. An HPLC chromatogram of a crude sample is shown in Figure 1. The retention times of all compounds shown in the HPLC chromatogram were 12.89

min for lactic acid, 14.17 min for formic acid, 15.35 min for acetic acid, and 16.85 min for 1,3-propanediol (internal standard).

The percent yield of lactic acid was calculated by the actual yield of lactic acid obtained divided by the theoretical yield of lactic acid based on the percentage of total carbohydrate in cassava starch (Table 1) as shown in the formula below. For simplicity, all carbohydrate content is assumed to be made up of anhydrous glucose units, and one anhydrous glucose unit could potentially convert into two lactic acid molecules.

$$\% \text{ yield lactic acid} = \frac{\text{Actual yield of lactic acid (mg)}}{\text{Theoretical yield of lactic acid based on available carbohydrates (mg)}} \times 100$$

Table 1. Proximate analysis of cassava starch.

Components	% content
Moisture	12.73
Lipid	0.26
Protein	0.00
Ash	0.14
Carbohydrate	86.90

## 3. Results and Discussion

The proximate analysis of cassava starch is shown in Table 1. A high carbohydrate content of 86.90% was found in the cassava starch, which was similar to a study that reported 87.8% carbohydrate content in cassava starch (Olufunmilola O., Ogugua C., Bussie, Ochuko L., & Gloria N., 2014). The other components in cassava starch were water and a trace amount of lipid (0.26%). Therefore, cassava starch could be a suitable carbohydrate feedstock for the hydrothermal conditions where its monosaccharide component would be converted into lactic acid.

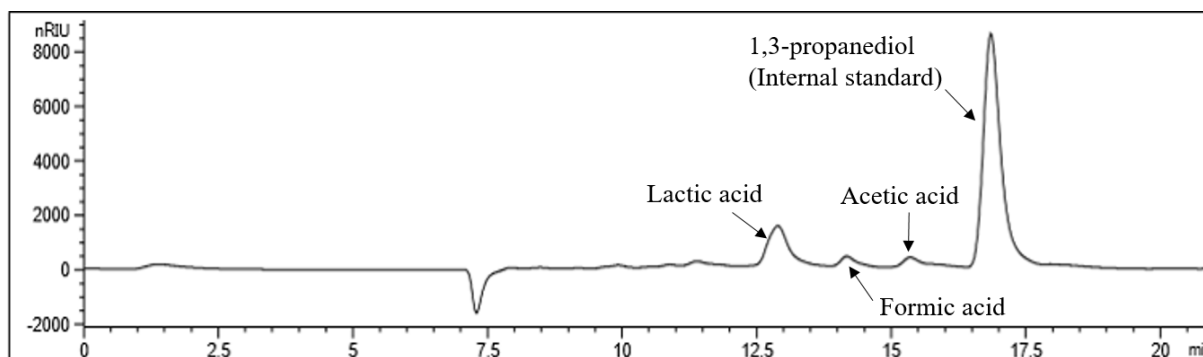


Figure 1. HPLC chromatogram of compounds in a crude sample, including lactic acid, formic acid, acetic acid, and 1,3-propanediol (internal standard), using Agilent 1260 series with VertiSep™ OA column, flow rate of 0.6 mL/min, and 0.01 N H<sub>2</sub>SO<sub>4</sub> as the mobile phase.

The chemical mechanism of the conversion of cassava starch to lactic acid starts with the hydrolysis of the polysaccharide starting material. Under alkaline hydrothermal conditions, cassava starch is hydrolyzed into monosaccharide units which are mainly glucose. The monosaccharide, a 6-carbon sugar, serves as a starting material for the Cannizzaro reaction pathway to give the desired product, lactic acid **6** (Scheme 1) (Ellis & Wilson, 2002). Initially, 6-carbon monosaccharide **1** is broken down into 3-carbon carbanion **2** and aldehyde **3**. After that, the protonation of carbanion **2** and the enolization of aldehyde **3** generate compound **4** which undergoes water  $\beta$ -elimination to form pyruvaldehyde **5**. Lactic acid **6** is then formed from the rearrangements of pyruvaldehyde **5** through hydride migration (a) or methyl migration (b).

### 3.1 Screening of base catalyst types

While divalent base  $\text{Ba}(\text{OH})_2$  was reported to be the most suitable catalyst for alkaline hydrothermal reaction of glucose to lactic acid (Esposito & Antonietti, 2013), KOH was also reported as the best catalyst for the alkaline hydrothermal reaction of glycerin to produce lactic acid (Shen *et al.*, 2009). Since many studies reported different efficient results of bases in catalyzing the alkaline hydrothermal reactions of biomass into lactic acid, a series of experiments were conducted by choosing divalent bases  $\text{Ba}(\text{OH})_2$ ,  $\text{Ca}(\text{OH})_2$ , and  $\text{Mg}(\text{OH})_2$ , and monovalent bases NaOH, and KOH as catalysts. The results are shown in Figure 2.

The results of this study demonstrated that  $\text{Ba}(\text{OH})_2$  is the most appropriate catalyst for the alkaline hydrothermal reaction which yielded  $30.6 \pm 0.7\%$  lactic acid. Esposito *et al.* also found that  $\text{Ba}(\text{OH})_2$  is the most effective catalyst to convert glucose to lactic acid by a hydrothermal reaction (Esposito & Antonietti, 2013). It was reported that a divalent base has a higher tendency to combine with carbonyl groups in the intermediate compound pyruvaldehyde to promote

lactic acid production (Shen *et al.*, 2009). Formic acid was obtained in a higher yield than acetic acid because formic acid is formed by the degradation of lactic acid and a direct conversion from the C-1 of glucose (Ellis & Wilson, 2002).

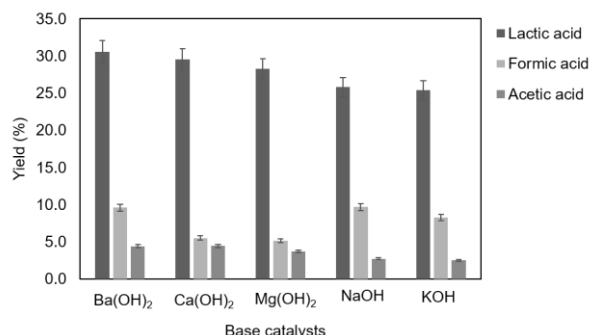
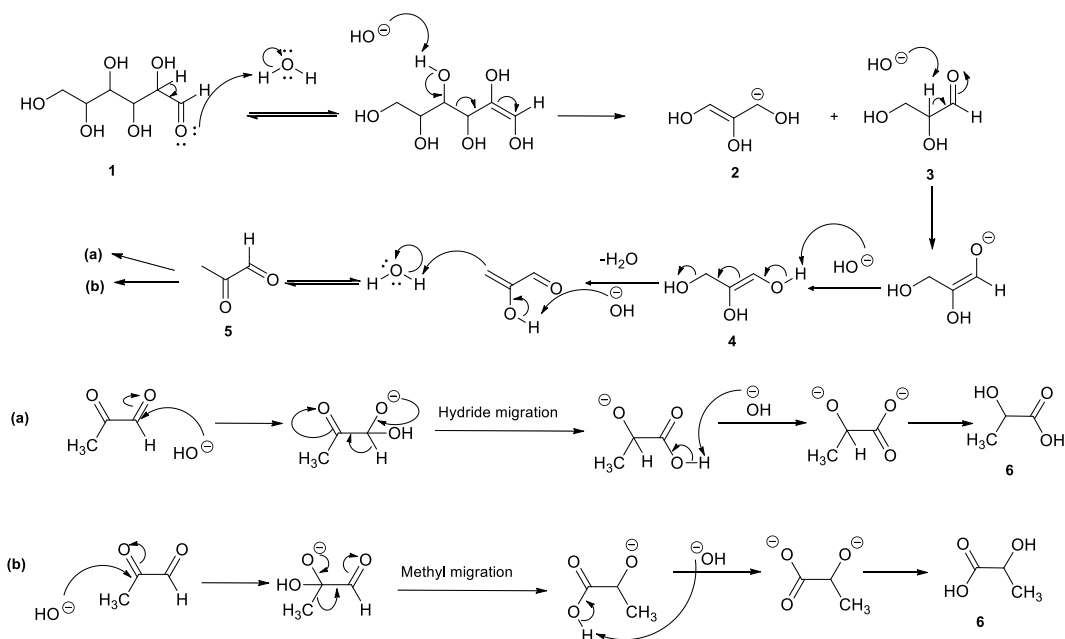


Figure 2. Results of lactic acid, formic acid, and acetic acid in the catalyst screening study using  $\text{Ba}(\text{OH})_2$ ,  $\text{Ca}(\text{OH})_2$ ,  $\text{Mg}(\text{OH})_2$ , NaOH, and KOH as catalysts (0.05 M) at  $240^\circ\text{C}$  for 30 min.

### 3.2 Effect of reaction temperatures on lactic acid yield

A set of experiments was performed to select a suitable temperature for the alkaline hydrothermal reaction of cassava starch into lactic acid. The results in Figure 3 reveal that a reaction temperature of  $240^\circ\text{C}$  provided the highest yield of lactic acid compared to the other reaction temperatures.

The highest yield of lactic acid at  $30.6 \pm 0.3\%$  was obtained at the reaction temperature of  $240^\circ\text{C}$ . For the reaction temperature range from  $180^\circ\text{C}$  to  $240^\circ\text{C}$ , the higher temperature can promote lactic acid production. A study reported that a reaction temperature higher than  $240^\circ\text{C}$  did not increase the lactic acid yield (Wang *et al.*, 2013).



Scheme 1. Cannizzaro reaction mechanism to convert glucose into lactic acid under hydrothermal conditions.

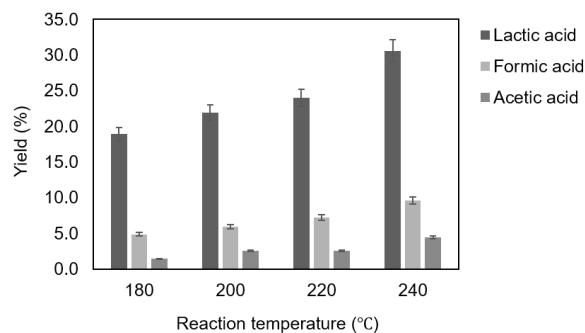


Figure 3. Reaction temperature effect on lactic acid, acetic acid, and formic acid yields from the hydrothermal reaction of cassava starch using  $\text{Ba}(\text{OH})_2$  at 0.05 M as the catalyst at various reaction temperatures of 180, 200, 220, and 240 °C for 30 min.

### 3.3 Effect of catalyst concentration on lactic acid yield

After the most effective catalyst and the most suitable temperature were determined, a set of experiments were performed to see the effect of catalyst concentrations on lactic acid yield. The results in Figure 4 illustrate that 0.15 M  $\text{Ba}(\text{OH})_2$  gave the highest lactic acid yield. As the concentration of  $\text{Ba}(\text{OH})_2$  was increased from 0.05 to 0.15 M, the lactic acid yields increased. However, at 0.2 M  $\text{Ba}(\text{OH})_2$  the product yield was lower than 0.15 M. The optimum concentration of 0.15 M  $\text{Ba}(\text{OH})_2$  gave the highest yield of lactic acid at  $34.7 \pm 0.9\%$ . Along with the increasing lactic acid yield, formic acid and acetic acid yields also increased because both acids were generated from the decomposition of lactic acid (Jin *et al.*, 2005). The formic acid yield increased and decreased in a similar fashion to the lactic acid yield because formic acid also decomposed into  $\text{CO}_2$  and  $\text{H}_2$  via a decarboxylation process under hydrothermal conditions (Yu & Savage, 1998).

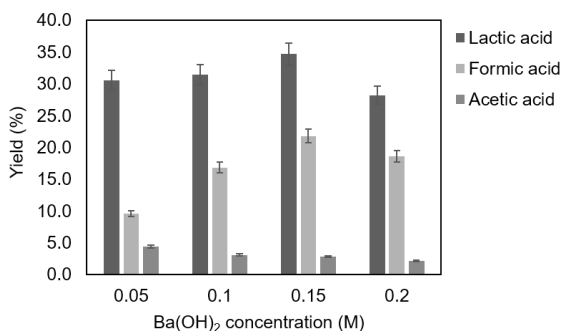


Figure 4. Lactic acid, acetic acid, and formic acid yields from the hydrothermal reactions of cassava starch at various concentrations of  $\text{Ba}(\text{OH})_2$ , 0.05, 0.1, 0.15, and 0.2 M at 240 °C for 30 min.

### 3.4 Reaction time effect on lactic acid yield

A set of experiments was conducted to determine the effect of time on the lactic acid yield. Two reaction times of 30 min and 60 min were chosen for the alkaline hydrothermal reaction of cassava with  $\text{Ba}(\text{OH})_2$  as the catalyst. The results are shown in Figure 5. A higher lactic

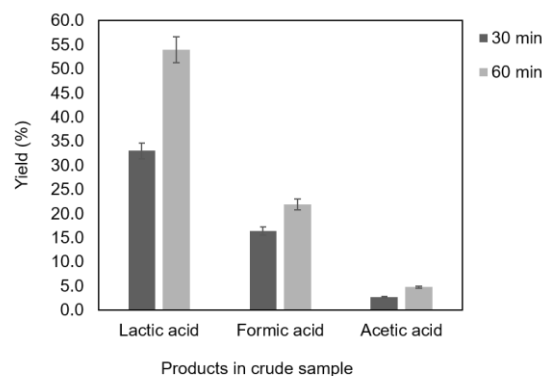


Figure 5. Effect of time on lactic acid, formic acid, and acetic acid yields from the hydrothermal reaction of cassava starch using 0.15 M  $\text{Ba}(\text{OH})_2$  at 240 °C for 30 and 60 min.

acid yield ( $53.95 \pm 0.8\%$ ) was obtained at the reaction time of 60 min. The trends of formic acid and acetic acid yields are also similar to that of the lactic acid, which increased at the longer reaction time. The results revealed that at the longer reaction time, cassava starch could be further hydrolyzed and decomposed which resulted in a higher yield of product.

As a result, the alkaline hydrothermal reaction of cassava starch with 0.15 M  $\text{Ba}(\text{OH})_2$  provides  $53.95 \pm 0.8\%$  lactic acid at 240 °C after 60 min. In comparison to previous studies, the lactic acid yield obtained in this study was much higher than previously reported (12%-27%) from potato starch at 300 °C (Jin & Enomoto, 2009; Jin *et al.*, 2005; Yan *et al.*, 2010). In this study, the reaction conditions for the highest yield of lactic acid from the alkaline hydrothermal process of cassava starch under microwave radiation were milder than the best reaction conditions reported in previous studies using a hydrothermal reaction. Thus, the conditions developed in the current study would not require a highly sophisticated reactor to sustain higher pressures and temperatures. This process would also consume less energy.

Under these hydrothermal conditions with 200 mg of cassava starch in 20 mL of reaction volume, the concentration of the lactic acid generated was  $4.95 \text{ gL}^{-1}$ . The mass balance yield (the ratio of lactic acid mass/cassava starch mass) was  $0.49 \text{ gg}^{-1}$  and the volume metric yield was  $4.95 \text{ gL}^{-1} \text{ h}^{-1}$ . In order to evaluate the possibility to increase the volume metric yield (Sirisansaneeyakul, 2013), the reactions were carried out at higher cassava starch concentrations of 1000 mg and 2000 mg in 50 mL of reaction volume. At 1000 mg/50 mL, the volumetric yield was higher at  $7.12 \text{ gL}^{-1} \text{ h}^{-1}$  and the concentration of lactic acid was higher at  $7.12 \text{ gL}^{-1}$ . At 2000 mg/50 mL, the volumetric yield was also higher at  $9.75 \text{ gL}^{-1} \text{ h}^{-1}$  and the concentration of lactic acid was higher at  $9.75 \text{ gL}^{-1}$ . Expectedly, the mass balance yields dropped to  $0.36 \text{ gg}^{-1}$  for the 1000 mg/50 mL reaction and to  $0.24 \text{ gg}^{-1}$  for the 2000 mg/50 mL reaction.

In comparison, the mass balance yields of lactic acid productions from the fermentation of cassava starch obtained under various optimal fermentation conditions were around 0.48 to  $0.50 \text{ gg}^{-1}$  (Chookietwattana, 2014; Xiaodong *et al.*, 1997; Yuwono *et al.*, 2011). The optimal hydrothermal conditions found in this study (0.15 M  $\text{Ba}(\text{OH})_2$ , 240 °C, 60 minutes) gave lower yields at 0.24 to  $0.49 \text{ gg}^{-1}$ . However, when the volumetric productivities and the much faster

operating time (60 min) are considered, the hydrothermal reaction contributes greatly to higher volumetric productivities of the chemical method. The aforementioned fermentations gave volumetric yields of 0.55 gL<sup>-1</sup>h<sup>-1</sup> to 1.88 gL<sup>-1</sup>h<sup>-1</sup> while the volumetric productivities were higher at 4.94 gL<sup>-1</sup>h<sup>-1</sup> to 9.74 gL<sup>-1</sup>h<sup>-1</sup>. The fermentation method also requires expensive protein supplement and culture media. Thus, the alkaline hydrothermal reaction of cassava starch is an effective method for producing lactic acid from a sustainable and low cost material in a short reaction time with a satisfactory lactic acid yield.

#### 4. Conclusions

Cassava starch is a suitable feedstock for lactic acid production by hydrothermal reaction due to its rich carbohydrate content and availability. Due to the advantage of a shorter operating time required by the alkaline hydrothermal reaction of cassava starch, the volumetric productivities obtained from the optimal hydrothermal conditions in this study were higher than the fermentation methods. The hydrothermal reaction, however, gave lower lactic acid mass per cassava mass ratio. The reaction conditions generated a small amount of formic acid and acetic acid, which is a benefit in lactic acid purification. In addition, the catalyst cost for the hydrothermal reaction is lower than the media and nutrient supplement costs required in a fermentation process. Thus, lactic acid production from the alkaline hydrothermal reaction of cassava starch is an efficient and applicable chemical technique that provides a satisfactory product yield.

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