ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Utilization of pineapple waste hydrolysate for lipid production by oleaginous yeast *Rhodoturula glutinis*

Jidapha Tinoi[a*] and Nuansri Rakariyatham[a]

[a]Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

Manuscript Info

Manuscript History:

Received: 15 January 2015
Final Accepted: 25 February 2015
Published Online: March 2015

Key words:

Pineapple waste, *Rhodotorula glutinis*, Acid hydrolysis, Lipid production, Oleaginous yeast

Corresponding Author

Jidapha Tinoi

Abstract

The oleaginous yeast *Rhodotorula glutinis* TISTR5159 performed the utilization of pineapple waste hydrolysates for growth and lipid accumulate. Pineapple waste hydrolysates were prepared with hydrothermal pretreatment and dilute sulfuric acid hydrolysis. Along with the high content of hemicellulose and cellulose, pineapple waste can be hydrolyzed into fermentable sugars. The results demonstrated the high yields of xylose (12.3 g/L) and glucose (10.7 g/L) in pineapple waste acid hydrolysate. Thus, pineapple waste acid hydrolysate presented the highest yield of cell dry weight (7.28 g/L) and lipid concentration (3.4 g/L or 46.7%). The fatty acid profile of *R. glutinis* lipid were dominated by long chain fatty acids, in particular C16 and C18 (palmitic acid, stearic acid, oleic acid and linoleic acid). Lipid produced by *R. glutinis* could be a valuable alternative raw material for the biodiesel production

1. Introduction

Pineapple is a fruit that widely cultivated in many tropical countries. Thailand is recently the largest producer of fresh pineapple in the world, following Philippines and Brazil, respectively. In 2014, 2.5 million tons of pineapple fruits were produced while as 9.5 million tons from the global. Of that amount, 80% of pineapples were processed in pineapple processing industry as canned pineapple and pineapple juice and 20% of them were consumed as fresh fruit. In addition, Thailand has been also exported pineapple around the world with as canned pineapple (640,000 tons) and pineapple juice (140,000 tons) with the value of 20,000 and 6,000 million baht, respectively. During pineapple processing, pineapple waste consists of peel, core, and residue pulp, have occurred in large amount. Therefore, increasing of pineapple production, pineapple wastes are also generated increase. The pineapple wastes are used for ruminant feed and disposed to the soil as a waste. Since, they are rich in carbohydrate and other nutrients. The main components of pineapple waste are cellulose, hemicellulose and lignin. Thus, like any other lignocellulosic feedstocks, pineapple waste can be converted to lipid after pretreatment and hydrolysis. In the previous research, pineapple wastes have a potential use as raw material for conversion to the high value-added products such as citric acid, lactic acid, ethanol, methane, proteolytic enzyme (Bromelain), bioactive compounds (Imandi et al. 2008, Tanaka et al. 1999, Abdullah, 2007,Ketnawa et al. 2012, Nigam, 1999).

Oleaginous microorganisms (microbial oil), are some microorganisms that accumulate lipid in their cell more than 20% of a dry biomass and also known as single cell oil. The lipids that accumulate inside their cell are formed oil droplet, that rich in polyunsaturated fatty acids or have specific structure (Kavadia et al. 2001). Recently, microbial oil can be used as a potential feedstock for biodiesel production, due to their chemical composition (fatty acids) are similar to common vegetable oil (soybean oil, palm oil). The advantages of lipid production by oleaginous microorganisms, their cultivation are affected neither by seasons nor by climates. In addition, they can produce and

accumulate lipid within a short time and grow well on a variety of substrates, even agricultural and agro-industrial wastes. A number of an oleaginous microorganism belonging to the genera of bacteria, yeast, fungi and microalgae, have the ability to produce lipids under their specific cultivation condition (Subramaniam et al. 2010). Among them, oleaginous yeast has more advantages for lipid production than algae, due to fast growth, high lipid content and can be utilized different substrates in lipid production (Li et al. 2008). Some oleaginous yeast strains, *Rhodotolura* sp., *Rhodosporidium* sp., *Yarrowia* sp., *Cryptococcus* sp., *Trichosporon* sp., *Lipomyces* have been reported that they can accumulate intracellular lipids up to 60% of their cell weight when glucose is used as substrate (Hu et al. 2009; Ageitos et al. 2011). Moreover, these oleaginous yeasts could be used agro - industrial waste as substrate for growth and lipid accumulation, even non-pretreated and pretreated agro-industrial wastes (Leiva-Candia et al. 2014).

Non - pretreated agro-industrial wastes such as crude glycerol from biodiesel production, monosodium glutamate wastewater, have been reported that they can be applied for lipid production. Saenge et al. (2011) demonstrated that oleaginous red yeast *R. glutinis* showed a potential use of crude glycerol from biodiesel plant for conversion to lipid and carotenoids. In addition, Chatzifragkou et al. (2011) presented that *Rhodotorula* sp. LFMB 22 was able to grow on glycerol waste and accumulate lipids while *Yarrowia lipolytica* LFMB19 comprised more than 80% of neutral lipids that suitable for biodiesel preparation. In the utilization of pretreated agricultural and agro-industrial wastes, lignocellulosic raw materials, are not directly hydrolyze by oleaginous yeasts, so their need to pretreated in order to produce fermentable monomers. Pretreatment methodologies can be categorized into physical, chemical and biological treatments or any combination of them (Chiaramonti et al. 2012). Lignocellulosic materials have been carried out for lipid production. Generally, they are composed of 35-55% cellulose, 20-40% hemicellulose and 10-25% lignin. Utilization of lignocellulosic materials as substrates for lipid production, the pretreatment is required to hydrolyse cellulose and hemicellulose into fermentable sugars, C5 and C6 sugars. In during pretreatment of lignocellulosic materials, the degradable by-products or inhibitors (acetic acid, furfural, 5-ydrolyfurfural etc.) were generated simultaneously, however the toxicity of these compounds is depend on their concentration. The potential uses of lignocellulosic hydrolysate for microbial oil production have been investigated (Yang et al.2014). The oleaginous yeast *Cryptococcus curvatus* ATCC20509 grow and accumulate lipid in the non-detoxified sweet sorghum bagasse hydrolysate that pretreated by microwave with lime followed by an enzymatic hydrolysis (Liang et al. 2012). Tsigie et al. (2011) demonstrated the sugarcane bagasse acid hydrolysate to provide an economically alternative carbon source for growth and lipid production of *Yarrowia lipolytica* Po1g. Corncob hydrolysate was investigated on growth and lipid production by *Trichosporon cutaneum* with two different pretreatment processes, enzyme and acid hydrolysis. Huang et al. (2012) hydrolysed corncob by *Tricoderma reesei* cellulase for using as substrate for *T. cutaneum* growth and reported that this strain is a promising for microbial oil production from lignocellulosic biomass, while Chen et al. (2013) evaluated the medium composition and fermentation on the microbial oil production by *T. cutaneum* on corncob acid hydrolysate. Other lignocellulosic materials such as rice straw, wheat straw, rapeseed meal, serum latex etc. were also applied for lipid production by oleaginous yeast. Rice straw was first performed for microbial oil production with treated by sulfuric acid hydrolysis. Detoxified rice straw acid hydrolysate gave a higher lipid yield than non-detoxified hydrolysate by *T. fermentans* (Huang et al. 2009). The hydrolysate from the dilute sulfuric acid pretreatment of wheat straw was explored for lipid production of five oleaginous yeasts, *C. curvatus, R. glutinis, Rhodosporidium toruloides, Lipomyces starkeyi* and *Y. lipolytica* (Yu et. al. 2011). The results showed that all of them could be used the detoxified hydrolysate to produce lipid while except *R. toruloides* could also use non-detoxified hydrolysate.

The purpose of this research is to investigate the capability of oleaginous yeast *Rhodotolura glutinis* TISTR5159 to produce lipids from pineapple waste hydrolysates with pretreated by hydrothermal and dilute sulfuric acid hydrolysis. Furthermore, the fatty acid profiles were characterized and transesterified into fatty acid methyl esters for the possibility to use yeast lipid in biodiesel preparation.

2. Material and Methods

2.1 Pineapple waste

Pineapple waste is the pulpy waste material that obtained as a residue from juice extraction of the fresh fruit which supplied by The Siam Agro Industry Pineapple and Others Co., Ltd., SAICO (Rayong, Thailand). The pineapple waste was dried at 60°C in a hot-air oven and ground to dried powder with passed through a 60 mesh screen. This material was used for all experiments. The characterization assays were performed following the Association of Official Analysis Chemistry (AOAC) for biomass analysis. The chemical compositions of pineapple waste were evaluated by using detergent fiber analysis to estimate the cellulose, hemicellulose and lignin.

2.2 Preparation of pineapple waste hydrolysates

The hydrothermal pretreatment and dilute sulfuric acid hydrolysis were selected to prepare the pineapple waste hydrolysates. Fig. 1 shows the flow diagram for hydrolysis process of pineapple waste. Hydrothermal pretreatment,

was performed by adding distilled water to solid with a ratio of 1: 10 (w/v) and treated by autoclaving at 121°C for 20 min. After cooling, pineapple waste hydrolysate was separated by centrifugation and vacuum filtration and then stored at 4°C until use. The dilute sulfuric acid hydrolysis was also carried out for pineapple waste hydrolysate preparation. Dried pineapple waste was mixed with 1.0%)v/v) sulfuric acid at a solid to liquid ratio of 1: 10 (w/v). The mixture was then autoclaved at 121°C for 20 min. After that, the mixture was centrifuged and neutralized to pH 6.0 with 6 M NaOH and then stored at 4°C. The recovered of total reducing sugar in pineapple waste acid hydrolysates were conducted.

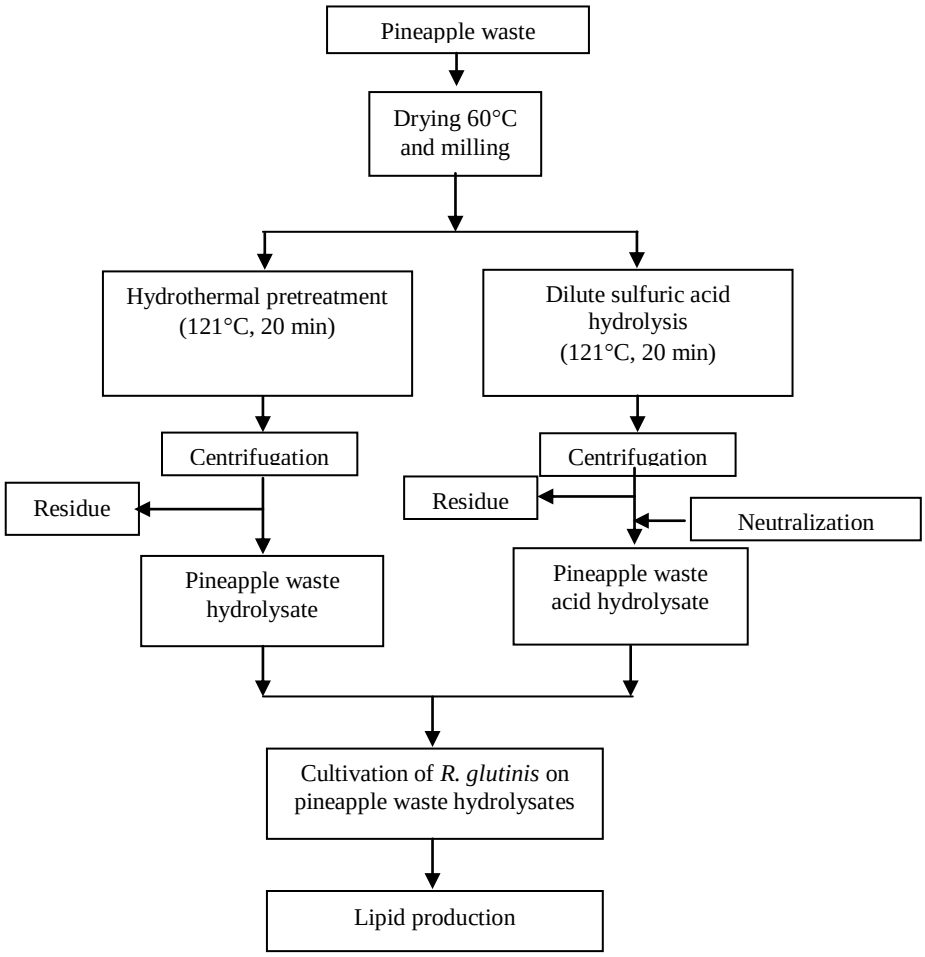

Fig. 1 Flow chart of the process to convert pineapple waste into lipid

2.3 Microorganism and preparation of pre-culture

Rhodotorula glutinis TISTR 5159 was obtained from the Thailand Institute of Science and Technological Research (TISTR) and maintained on Yeast – Malt (YM) medium at 4^{o}C. The pre-culture was grown in 125 mL Erlenmeyer flask containing 30 mL of YM broth (glucose 10 g L^{-1}, yeast extract 3 g L^{-1}, malt extract 3 g L^{-1} and peptone 5 g L^{-1}) for 36 h at 30°C on a rotary shaker (160 rpm).

2.5 Lipid production by *R. glutinis* TISTR5159 cultured in pineapple waste hydrolysates

Incula (10%, v/v) were inoculated into 250 ml Erlenmeyer flask contained 90 ml of sterilized pineapple waste hydrolysate. The cultures were incubated at 30°C on a rotary shaker at 160 rpm for 168 h. Samples were taken at 12 h time intervals to determine the cell dry weight, residual reducing sugar and lipid content. All experiments were performed in triplicate.

2.6 Analytical methods

2.6.1 Cell dry weight determination

The cell dry weight (expressed as g/L) was determined by gravimetrically. The 10 ml of cell suspensions were centrifuged at 4,000 rpm for 10 min and the cell pellet were then washed twice with distilled water, dried at 105^{o}C for 3 h and weighed until constant.

2.6.2 The total reducing sugar determination and sugar analysis

Total reducing sugar concentration was determined according to the DNS colorimetric method and the results were calculated on calibration curves of standard D-glucose. The concentration of glucose, fructose, xylose, arabinose in pineapple waste hydrolysate were determined by HPLC (Hewlett Packard Agilent 1100 series) equipped with Alltech® Econosphere^{TM} NH_2 column (5 μm particle size, 4.6 mm x 250 mm) and RI detector. The mixture of acetonitrile: water (90:10, v/v) was used as mobile phase with a flow rate 1.2 mL/min.

2.6.3 Acetic acid, furfural and 5-hydroxymethylfurfural (HMF) and fatty acid methyl ester analysis

Acetic acid, furfural and 5-hydroxymethyl furfural in pineapple waste hydrolysates and fatty acid methyl ester were determined by using a gas chromatography GC-17A (Shimadzu, Japan) equipped with DB-1 capillary column (0.25 mm x 30 m) and a flame ionization detector. The column temperature was programmed from 150^{o}C to 260^{o}C at a rate of 6^{o}C/min. The injector and the detector temperature were set at 260^{o}C and 300^{o}C, respectively. Nitrogen gas was used as carrier gas at 0.80 mL/min. The fatty acid profile of oil produced by *R. glutinis* yeast was prepared by methylation for the conversion of fatty acids to fatty acid methyl esters (FAME) according to the method of Morrison and Smith (1964). Fatty acids were methylated by boron trifluoride in methanol.

2.6.2 Lipid extraction and determination

Lipid extraction was performed according to the modified of Bligh and Dyer (1959). Lipid was extracted from the lyophilized cell by using a mixture of chloroform: methanol (2:1, v/v) for 20 min. The extracted lipid was centrifuged to obtain clear supernatant and the same process was repeated about three times. The whole extracted lipid in solvent phase was collected and sodium sulfate anhydrous was added to remove any residue moisture. The solvent was evaporated and dried under vacuum. Total lipid was estimated by gravimetric method.

2.7 Statistical analysis

All experiments were carried out in a completely randomized design. The results were subjected to analysis of variance (one-way ANOVA) and the treatment means were compared using the least significant difference (LSD) values at a significance level of $P<0.05$.

3. Results and discussion

3.1 Chemical composition of pineapple waste and pineapple waste hydrolysate

Table 1 shows the proximate composition of dried pineapple waste. The total protein, fat and ash contents of pineapple waste were 6.80, 1.35 and 3.8 % (w/w, dry basis). The result also presented the high content of total carbohydrate (75.6±0.60%) which that consist of crude fiber (68.4±0.50%). On the detergent fiber analysis of crude fiber in pineapple waste, cellulose, hemicelluloses and lignin were found to be 28.4±0.35%, 38.6±0.70% and 8.50±0.50%, respectively. According to Chaokaur et al. (2009) reported the evaluation of nutritive value and sugar soluble carbohydrate of pineapple residue and found that pineapple pulp contained cellulose (30.67%), hemicelluloses (39.92%) and lignin (8.17%). The high content of cellulose and hemicelluloses in pineapple waste were considered to be using as a promising carbon source for the production of microbial oil, especially oleaginous yeast. For the high potential to utilize the pineapple waste in lipid production, cellulose and hemicelluloses structures were hydrolyzed into small molecules such as monosaccharides (glucose, xylose, arabinose etc.) under the suitable pretreatment and hydrolysis processes.

Table 1 The proximate composition of pineapple waste on dry solid basis.

Composition	% (w/w, dry basis) *
Moisture	13.4 ± 0.42
Total Protein	6.80 ± 0.12
Fat	0.35 ± 0.20
Ash	3.80 ± 0.25
Total Carbohydrate	75.6 ± 0.60
- Crude fiber	68.4 ± 0.50
- Cellulose	28.4 ± 0.35
- Hemicellulose	38.6 ± 0.70
- Lignin	8.50 ± 0.50

* Mean and standard deviation values of triplicate determination.

The pretreatment and hydrolysis processes are necessary ways in the bioconversion of pineapple waste. To evaluate an effective of treatment methods for pineapple waste hydrolysis in lipid production by *R. glutinis* TISTR5159, the hydrothermal treatment and acid hydrolysis were carried out to prepare pineapple waste hydrolysate. The results show that the amounts of released sugar (g/L) were varied. Considering sugar liberated from cellulose and hemicelluloses of pineapple waste after pretreatment and hydrolysis processes (Table 2). The hydrothermal method with 1:10 of pineapple waste to water ratio had effected to intensity on the recovery of total soluble sugars. The concentration of total reducing sugar was found to be 2.5 g/L, including glucose (1.4 g/L) and fructose (0.8 g/L). Comparison to dilute sulfuric acid hydrolysis, is the chemical method that to cleavage the linkage between xylose residues of xylan backbone in hemicelluloses structure. The result shows the high content of total reducing sugar (27.7 g/L) was obtained from pineapple waste that treated by 1% sulfuric acid under an autoclaving condition for 20 min. The pineapple waste acid hydrolysate also contained the high xylose and glucose contents which of 12.3 and 10.7 g/L, respectively. Generally, for a favorable lipid production of oleaginous yeast *R. glutinis*, the excess of a carbon source and concurrently a limitation of nitrogen are needed (Ratledge, 2010). In addition, it can be assumed that the lipid production of the oleaginous yeast is enhanced with increasing total sugar content of the feedstock. In this study, pineapple waste acid hydrolysate was considered to be a suitable carbon source for oil production of *R. glutinis*.

The advantages of acid hydrolysis are fast, inexpensive, simple to operate, efficient, low energy consumption which obtained the high yield of reducing sugar, whiles the degradable by-products such as furfural, 5-hydroxymethylfurfural (HMF) and acetic acid were also generated. The high content of degradable products could be inhibited the growth of oleaginous yeast. But in this study, the pineapple waste acid hydrolysate contained the degradable products which found to be at low concentration. It had ineffective to the growth and oil production of oleaginous yeast especially *R. glutinis* TISTR5159. According to Yu et al. (2011) utilized the hydrolysate from dilute sulfuric acid pretreatment of wheat straw for microbial oil production, the results show that hydrolysate composed of pentose (xylose, arabinose) (24.3 g/L) and hexose (glucose, galactose)(4.9 g/L), along with some degradation products, such as acetic acid, furfural and 5-hydroxymethylfurfural and represented the insignificantly inhibition on biomass and oil production at concentration of up to 3 g/L in non-detoxified wheat straw hydrolysate. The possibility of utilizing sugarcane bagasse hydrolysate for microbial oil production was carried out by Tsigie et al. (2011). Sugarcane bagasse hydrolysis with 2.5% HCl resulted in maximum total sugar content (21.38 g/L) with xylose, glucose and arabinose consisted of 13.59, 3.98 and 2.78 g/L, respectively. The 5-hydroxymethyl furfural (0.61 g/L) and furfural (0.12 g/L) were also obtained. While as, the inhibitory effect of furfural and HMF were investigated and found that *R. glutinis* and *L. starkeyi* grew only at furfural concentration less than 1 g/L, but *T. cutaneum* showed the relatively better tolerance to furfural. 5-HMF presented the inhibition of cell growth and lipid production weakly in the tested concentration (0.5 – 2 g/L)(Chen et al. 2009).

Table 2 Chemical compositions of pineapple waste hydrolysates.

Chemical composition	**Concentration (g/L)***	
	Pineapple waste hydrolysate	
	Hydrothermal pretreatment	**Dilute sulfuric acid hydrolysis**
Total reducing sugar	2.5±0.2	27.7±0.35
Total Nitrogen	0.14±0.35	0.24±0.15
Glucose	1.4±0.52	10.7±0.32
Fructose	0.8±0.25	3.5±0.40
Xylose	-	12.3±0.20
Arabinose	-	0.63±0.30
Furfural	-	0.32±0.20
Hydroxymethylfurfural	-	0.15±0.40
Acetic acid	-	0.25±0.25

*Mean and standard deviation values of triplicate determination.

3.2 The growth and lipid production of *R. glutinis* TISTR5159 on pineapple waste hydrolysates

The growth and lipid production of *R. glutinis* TISTR5159 on pineapple waste hydrolysates that obtained from hydrothermal pretreatment and dilute sulfuric acid hydrolysis were shown in Table 3. The *R. glutinis* was able to grow on all of the pineapple waste hydrolysates and produced lipid as a product. The results show the significant different of growth and lipid production of *R. glutinis* among the pineapple waste hydrolysates. The results indicated that *R. glutinis* was the most significant growth when cultured on pineapple waste acid hydrolysate which cell dry weight of 4.28 g/L. Because pineapple waste acid hydrolysate contained high sugar content which *R. glutinis* could be used as carbon source for cell proliferation. While *R. glutinis* also exhibited the ability to grow in pineapple waste hydrolysate obtained from hydrothermal pretreatment. For lipid production in pineapple waste hydrolysates, the results evaluated that the highest lipid content (36.7%) with lipid concentration (1.57 g/L) and lipid productivity (0.37 g/g dry cell) when cultured in pineapple waste acid hydrolysate. This research represents the possibility of lipid production on pineapple waste hydrolysate. *R. glutinis* is capable of assimilate both hexose (glucose) and pentoses (xylose and arabinose) in pineapple waste acid hydrolysate. The lipid production of *R. glutinis* was unaffected by acetic acid, furfural and 5-hydroxyl methylfurfural (HMF). The possible reasons were the high sugar concentration which led to high cell biomass and lipid accumulation, in addition, *R. glutinis* could tolerant the inhibitor of these concentrations in pineapple waste acid hydrolysate.

Table 3 The growth and lipid production of *R. glutinis* TISTR5159 on pineapple waste hydrolysates.

Media	Cell dry weight (g/L)	Lipid concentration (g/L).	Lipid productivity (g/g dry cell)	Lipid content (%)	Residue reducing sugar (g/L)
Pineapple waste hydrolysate					
By hydrothermal pretreatment	4.71 ±0.20[b]	0.989 ±0.15[b]	0.210±0.18[b]	21.1±0.15[b]	0.02±0.10
By dilute sulfuric acid hydrolysis	7.28 ±0.22[a]	3.40 ±0.15[a]	0.467±0.10[a]	46.7±0.20[a]	0.12±0.20

[*] Mean and standard deviation values of triplicate determination.
[a-b] means the column followed by different letters are significantly different ($P < 0.05$)

This research presented the possibility for utilization pineapple waste hydrolysate as a carbon source for lipid production by *R. glutinis* TISTR5159, especially pineapple waste acid hydrolysate. Recently, there are many researchers reported the use of dilute sulfuric acid for hydrolysis of lignocellulosic materials. Hu et al. (2010) reported the dilute sulfuric acid hydrolysis can convert hot-water sugar maple wood hemicelluloses extract to sugars, resulting hydrolysate is rich in monomeric sugars, especially in xylose and can be used as fermentable media to produced bio-ethanol and other valuable products. Moreover, microbial oil production by *T. fermentans* from rice straw hydrolysate treated with sulfuric acid was performed and gave total biomass (8.6 g/L) and lipid content (11.5 g/L) after cultured on the detoxified rice straw hydrolysate.

3.3 Time curse of growth and lipid production on pineapple waste acid hydrolysate

Time curse of growth and lipid accumulation of *R. glutinis* on pineapple waste acid hydrolysate were evaluated in Fig. 2. *R. glutinis* could grow and produced lipid in the early phase of cultured on pineapple waste hydrolysate with relatively slow and then after cultivation for 24 h presented rapidly increased. Cell dry weight reached the maximum of 12.3 g/L while lipid content of 6.4 g/L or 52% when cultured for 132 h. This results indicated the increasing of lipid accumulate when cell growth enhanced. In the generally, the exhaustion of nitrogen source could be generated by oleaginous yeast to produce lipid when extra carbons are still available. Consumption of reducing sugar as well as lipid concentration in pineapple waste acid hydrolysate during fermentation also show in Fig.2. The results revealed that reducing sugar was utilized gradually increased with time after inoculation and found to be almost completely consumption of reducing sugar (5.3 g/L) at the end of fermentation. The lipid production of *R. glutinis* was increased when cultured in pineapple waste acid hydrolysate that prepared under the optimized condition and presented the lipid content enhanced from 36.7% up to 51% (w/w). Comparison of capability of *R. glutinis* for using lignocellulosic materials as substrate in lipid accumulation, *R. gultinis* can be produced lipid from pineapple waste

hydrolysate higher than from corn stalk (11.8%), tree leaves (28.6%) and rice straw hydrolysates (5.74%) (Dai et al. 2007). Moreover, this work also presented higher of lipid content than non-detoxified and detoxified liquid wheat straw hydrolysates which lipid of 25% and 20% (w/w), respectively when prepared by dilute acid hydrolysis (Yu et al. 2011). This work shows the high potential for using pineapple waste hydrolysate as a carbon source for lipid production by *R. glutinis* TISTR5159.

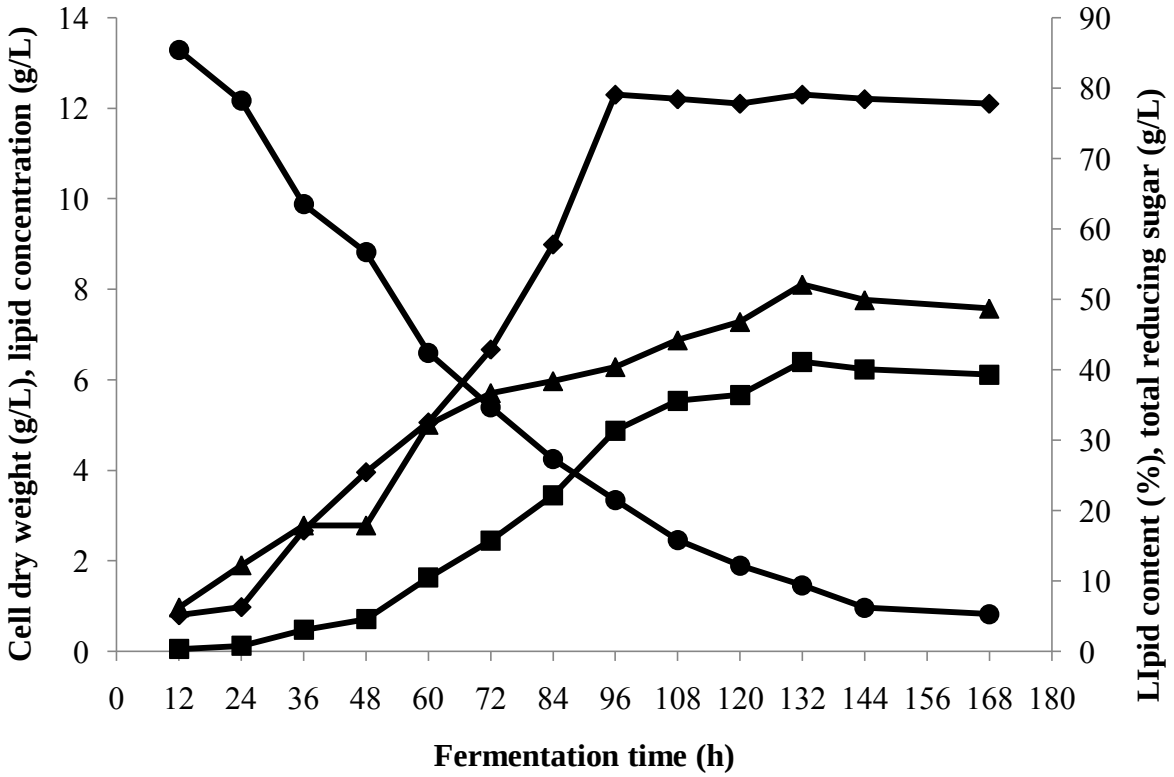

Fig. 2 Time course of growth and lipid production by *R. glutinis* cultured on pineapple waste acid hydrolysate.
◆ Cell dry weight, ■ Lipid concentration, ▲ Lipid content, ● Total reducing sugar.

3.4 Fatty acid compositions

Lipid that obtained from *R. glutinis* was transesterified and analyzed by GC analysis. The relative percentages of fatty acid methyl esters were calculated by measuring their peak areas. The lipid produced by *R. glutinis* was composed of long-chain fatty acid with 16 and 18 carbon atoms. It was found that those lipid mainly contained palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) as presented in Fig.3. All of the major fatty acids which accounted for more than 85% of the fatty acids in the sample were indicated. The results presented the composition of *R. glutinis* lipid was similar to that of vegetable oil and *R. glutinis* cultivated on different carbon sources such as glycerol, Switch grass acid hydrolysate, palm oil mill effluent, wheat straw and Miscanthus hydrolysate (Esaterling et al. 2009; Dai et al. 2007; Zhang et al. 2011; Saenge et al. 2011; Mast, at al., 2014). The fatty acid profile in lipid *R. glutinis* has been reported that it represented the high potential utility for biodiesel production (Dai et al. 2007, Li et al. 2010, Schneider et al. 2013).

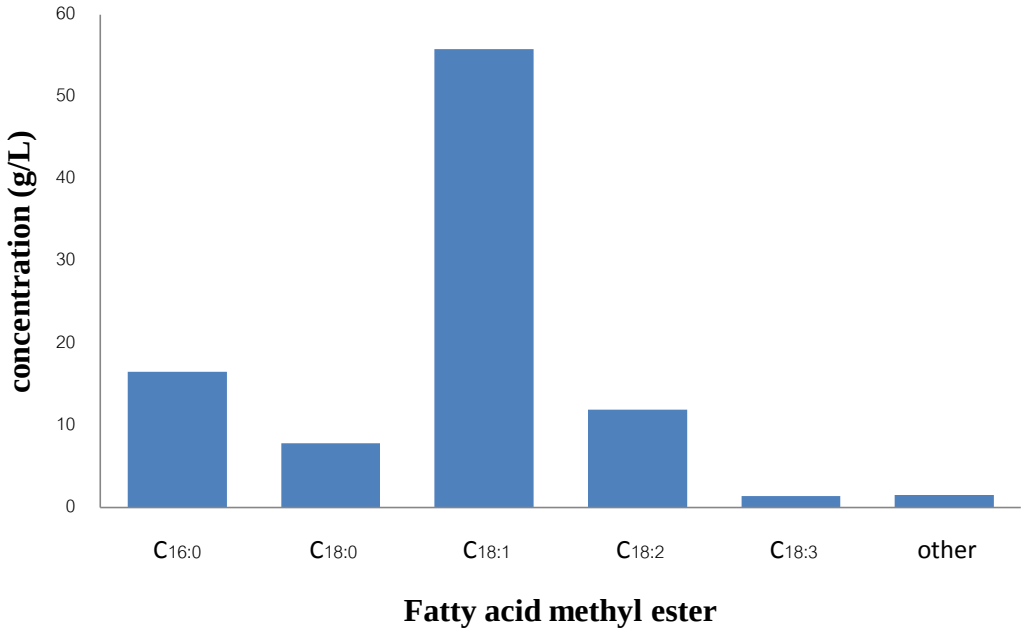

Fig. 3 Fatty acid composition (g/L) by *R. glutinis* grown on pineapple waste acid hydrolysate

4. Conclusion

This research demonstrated *R. glutinis* TISTR5159 is able to utilize the pineapple waste hydrolysate as a carbon source for growth and lipid production. Various treatments were applied to prepare the pineapple waste hydrolysates, acid hydrolysis presented highest content of sugar and also showed the highest of lipid content. Thus, pineapple waste could be used as inexpensive substrate for microbial oil production that further using in biodiesel production.

Acknowledgments

The authors acknowledge the financial support of The Thailand Research Fund (TRF) and Office of the Higher Education Commission (MUA) and grateful to Department of Chemistry, Faculty of Science, Chiang Mai University and Division of Biology, Faculty of Science, King Mongkut's Institute Technology Ladkrabang.

References

Abdullah, M., Sodarto, S.H., 2007. Solid and liquid pineapple waste utilization for lactic acid fermentation using *Lactobacillus delbrueckii*. Reaktor, 11, 50-52.

Agietos, J.M., Vallejo, J.A., Veiga-Crespo, P., Villa, T.G., 2011. Oily yeasts as oleaginous cell factories. Appl. Microbiol. Biotechnol. 90, 1219-1227.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Phys. 37, 911-917.

Chaokaur, A., Laikhonburi, Y., Kunmee, C., Santhong, C.,Chimthong, S., 2014. Evaluation of nutritive value and sugar carbohydrate of pineapple residue. Khon Kaen Agr. J. 42, 301-306.

Chen, X., Huang, C., Yang, X.,Xiong, L., Chen, X., Ma, L., 2013. Evaluating the effect of medium composition and fermentation condition on the microbial oil production by *Trichosporon cutaneum* on corncob acid hydrolysis. Bioresour. Technol. 143, 18 – 24.

Chiaramonti, D., Prussi, M., Ferreo, S., Oriani, L., Ottonello, P., Torre, P., Cherchi, F., 2012. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. Biomass Bioenerg. 46, 25-35.

Dai, C., Tao, J., Xie, F., Dai, Y., Zhao, M., 2007. Biodiesel generation form oleaginous yeast *Rhodotorula glutinis* with xylose assimilating capacity. Afr. J. Biotechnol. 6, 2130-2134.

Esterling, E.R., French, W.T., Hernandez, R., Licha, M. 2009. The effect of glycerol as sole and secondary substrate on growth and fatty acid composition of *Rhodotorula glutinis*. Bioresour. Technol. 100, 356-361.

Imandi, S.B., Bandara, V.V.R., Somalanka, S.R., Bandaru, S.R., Garapati, H.R., 2008. Application of statistical experimental designs for the optimization of medium constituents for the production of citric acid from pineapple waste. Bioresour.Technol. 99, 4445-4450.

Hu., C., Zhao, X., Zhao, J., Wu, S., Zhao, Z.K., 2009. Effects of biomass hydrolysis by-products on oleaginous yeast *Rhodosporidium toruloides*. Bioresour. Technol. 100, 4843-4847.

Hu, R., Lin, L., Liu, T., Liu, S., 2010. Dilute sulfuric acid hydrolysis of sugar maple wood extract at atmospheric pressure. Bioresour.Technol. 101, 3586-3594.

Huang, C., Zong, M., Wu, H., Liu, Q., 2009.Microbial oil production from rice straw hydrolysate by *Trichosporon fermentans*. Bioresour. Technol. 100, 4535 – 4538.

Huang, C., Chen, X., Xiong, L., Chen, X., Ma, L., 2012. Oil production by the yeast *Trichosporon dermatis* cultured in enzymatic hydrolysates of corncobs. Bioresour. Technol. 110, 711 – 714.

Kavadia, A., Komaitis, M., Chevalot, I., Blanchard, F., Marc, I., Aggelis, G., 2001. Lipid and gamma-linolenic acid accumulation in strains of Zygomycetes growing on glucose. J. Am. Oil chem. Soc., 78, 341-346.

Ketnawa, S., Chaiwut, P., Rawdkuen, S., 2012. Pineapple waste: A potential source for bromelain extraction. Food Bioprod. Precess. 90, 385-391.

Lei-Candia, D.E., Pinzi, S., Redel-Macías, M.D., Koutinas, A., Webb, C., Dorado, M.P., 2014.The potential for agro-industrial waste utilization using oleaginous yeast for the production biodiesel. Fuel. 123, 33 – 42.

Li, M., Liu, G-L., Chi, Z., Chi, Z-M., 2010. Single cell oil production from hydrolysate of cassava starch by marine-derived yeast *Rhodotorula mucilaginosa* TJY15a. Biomass Bioenerg. 34, 101 – 107.

Li, Q., Du, W., Liu, D.H., 2008. Perspectives of microbial oils for biodiesel production. Appl. Microbiol. Biotechnol. 80, 749-756.

Li, Y., Zhao, Z.K., Bai, F., 2007. High-density cultivation of oleaginous yeast *Rhodosporidium toruloides* Y4 in fed-batch culture. Enzym. Microb. Technol. 41, 312-317.

Liang, Y., Tang, T., Umagiliyage, A.L., Siddaramu, T., McCarroll, M., Choudhary, R., 2012. Utilization of sorghum bagasse hydrolysates for producing microbial lipids. Appl. Energy. 91, 451-458.

Nigam, J.N., 1999. Continuous ethanol production from pineapple cannery waste. J. Biotechnol. 72, 197-202.

Saenge, C., Cheirsilp, B., Suksaroge, T.T., Bourtoom, T., 2011. Efficient concomitant production of lipids and carotenoids by oleaginous red yeast *Rhodotorula glutinis* cultured in palm oil mill effluent and application of lipids for biodiesel production. Biotechnol. Bioprocess Eng. 16, 23-33.

Saenge, C., Cheirsilp, B., Suksaroge, T.T., Bourtoom, T., 2011. Potential use of oleaginous red yeast *Rhodotorula glutinis* for the bioconversion of crude glycerol from plant to lipids and carotenoids. Process Biochem. 46, 210 – 218.

Schnetder, T., Graeff-Hönninger, S., French, W.T., Hernandez, R., Merkt, N., Claupein, W., Hetrick, M., Pham, P., Lipid and carotenoid production by oleaginous red yeast *Rhodotorula glutinis* cultivated on brewery effluents. Energy. 61, 34-43.

Subramaniam, R., Dufreche, S., Zappi, M., Baipai, R., 2010. Microbial lipids from renewable resources : production and characterization. J. Ind. Microbiol. Biotechnol. 37, 1271 – 1287.

Tanaka, K., Hilary, Z.D., Ishizaki, A., 1999. Investigation of the utility of pineapple juice and pineapple waste material as low-cost substrate for ethanol fermentation by *Zymomonas mobilis*. J. Biosci. Bioeng. 87, 642-646.

Tsigie, Y.A., Wang, C, -Y., T, C, -T., Ju, Y, -H., 2011. Lipid production from *Yarrowia lipolytica* Po1g grown in sugarcane bagasse hydrolysate. Bioresour. Technol. 102, 9216-9222.

Xiaowei, P., Hongzhang, C., 2012. Hemicellulose sugar recovery from steam-exploded wheat straw for microbial oil production. Process Biochem. 47, 209 – 215.

Yang, T.C., Kumaran, J., Amartey, S., Maki, M., Li, X., Lu, F., Qin, W., 2014. Biofuels and bioproducts produced through microbial conversion of biomass. In: Gupta, V.K., Tuohy, M.G., Kubicek, C.P., Saddiler, J., Xu, F. (Eds), Bioenergy research: advance and application. Elsevier, UK, pp. 71-93.

Yu, X., Zheng,Y., Dorgan, K.M., Chen, S., 2011. Oil production by oleaginous yeasts using the hydrolysate from pretreatment of wheat straw with dilute sulfuric acid. Bioresour. Technol. 102, 6134 – 6140.

Zhang, G., French, W.T., Hernandez, R., Alley, E., Paraschivescu, M., 2011. Effects of furfural and acetic acid on growth and lipid production from glucose and xylose by *Rhodotorula glutinis*. Biomass Bioenerg. 35, 734-740.