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Cultivation and Lipid Production of Oleaginous Yeast on Canteen Food Waste

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ABSTRACT

Food waste is the organic waste that is attractive for using as raw material in value-added bioproducts production via fermentation process. Canteen food waste was collected and determined the chemical composition. It mainly consisted of carbohydrate (49.24% dry basis, w/w) and subjected as feedstock for fermentable sugar and lipid production. The commercial α-amylase and glucoamylase were used for hydrolysis of 30% solid loading of food waste and presented total reducing sugar concentration of 169.13±5.67 g/L with a high glucose content (77.83% of total sugars). The oleaginous yeasts (*Rhodotorula glutinis* TISTR 5159, *Yarrowia lipolytica* TISTR 5621 and *Rhodosporidium toruloide*s TISTR 5123) were cultivated on food waste hydrolysate. The results presented that *R. glutinis* TISTR 5159 and *Y. lipolytica* TISTR 5621 were an efficient and feasible in growth and lipid production on food waste hydrolysate containing 20 g/L of reducing sugar concentration. *Y. lipolytica* TISTR 5621, the highest biomass (3.95 g/L) and lipid content (12.82% of dry biomass) were achieved at 96 h of cultivation. The highest biomass and lipid content of *R. glutinis* TISTR 5159 were 5.91 g/L (at 384 h of cultivation) and 11.01% of dry biomass (at 288 h of cultivation), respectively. The lipid of *Y. lipolytica* TISTR 5621 was consisted of C16 and C18 as high as 99% of total fatty acid which could be an alternative feedstock for value-added products production.

Keywords: Canteen food waste, Enzymatic hydrolysis, Oleaginous yeast, Lipid production, Fatty acid composition

INTRODUCTION

Food waste usually defines as edible material that is subsequently discarded and lost (Girotto et al., 2015). Food waste generation by the end-consumers is occurred due to bad consumption behavior, over-preparation and over-purchasing (Schanes et al., 2018). The non-effective of food waste treatment can be caused of many negative impacts on environmental level such as air pollution, bad odor, leachate and greenhouse gas emission (Karmee and Lin., 2014). Thus, the sustainable of food waste treatment is needed to be carried out.

Food waste is contained the significant amount of organic substances such as carbohydrate, protein and lipid. The chemicals composition of food waste are varied widely depending on resource. According to Pleissner et al., (2013) presented that canteen food waste (Hong Kong) containing rice, noodles, meat, and vegetables contained carbohydrate (33%), lipid (15%), protein (10%) and phosphate (3.9%). While kitchen waste from food court (Turkey) was determined and found that carbohydrate, protein and lipid were 60%, 4.5%, and 8.8%, respectively (Cekmecelioglu and Uncu 2013). In addition, Hafid et al., (2015) reported that the main composition of cafeteria kitchen (Malaysia) waste consisted of carbohydrate (60%), protein (20%) and lipid (13%).

Food waste can be hydrolyzed for nutrients recovery. The enzymatic hydrolysis method is widely subjected for sugar recovery from carbohydrate-rich food waste because of it is simple and environmentally friendly. Yan et al. (2011) conducted the response surface methodology for the optimization of α-amylase and glucoamylase hydrolysis in sugar production from food waste. The reducing sugar concentration was obtained as high as 164 g/L. Additionally, food waste hydrolysate containing high reducing sugar concentration of 194.43 g/L was obtained via fed batch enzymatic (α-amylase and glucoamylase) saccharification (Yan et al., 2012). Cekmecelioglu and Uncu (2013) obtained high glucose yield (65 g /100 g of food waste) from two steps enzymatic hydrolysis of food waste which performed by the liquefaction (α-amylase) and saccharification (glucoamylase, cellulase, and β-glucosidase).

Food waste is the promising feedstock for various value-added products production such as lactic acid, biogas, bioethanol and biolipid (Carneiro et al., 2008; Kim et al., 2011; Hao et al., 2015; Kwan et al., 2016). The lipid obtained from food waste or waste cooking oil has been subjected for biodiesel production because of it is non-value raw material and non-competitive with edible food stuff (Karmee, 2016). The solid part of food waste rich in nutrients

can be utilized as feedstock for lipid production by oleaginous yeast cultivation. Many oleaginous yeast such as *Rhodotorula glutinis*, *Yarrowia lipolytica*, *Rhodosporidium toruloides*, etc. have been reported as the potential lipid producers with vary lipid content of 26 to 53% of dry biomass (Hao et al., 2015; Johnravindar et al., 2018; Ma et al., 2018). The oleaginous yeast lipid has the fatty acid composition similar to plant oil or animal fat which can be an available lipid feedstock for value-added products production such as dietary supplements, cocoa butter substitutes, biodiesel, etc. (Meng et al., 2009; Papanikolaou and Aggelis, 2011; Thevenieau and Nicaud, 2013).

This research aims to utilize the food waste hydrolysate obtained from enzymatic hydrolysis as low-cost feedstock for oleaginous yeast cultivation and lipid production.

MATERIALS AND METHODS

Materials

Raw material. Food waste was collected from canteen at Department of Biology, Faculty of Science, Chiang Mai University (Thailand). Bones, plastics, tissue paper and other inorganic materials were separated out. Food waste was pre-dried in hot air oven at 60 °C and blended into small pieces by using blender. Blended/pre-dried food waste with particle size of 12 mesh was then dried at 60 °C until constant weight. Dried food waste was kept in plastic bag at room temperature for further experiments.

The chemical compositions of food waste (dry basis) were analyzed based on the methods of AOAC (1990). Lipid content was determined by Soxhlet extraction method. Crude protein and nitrogen content were estimated using Kjeldahl method (conversion factor of 6.25). Moisture content was determined by drying in hot air oven at 105 °C until constant weight. The total ash content was determined by the combustion at 600 °C for 3 h. The fiber content was determined by acid base digestion and solubilization. The remaining percentage was assumed to be the total carbohydrate content.

Enzymes. Two commercial enzyme solutions, *Bacillus licheniformis* α-amylase type XII-A (Sigma-Aldrich, United States) with an enzyme activity of 156,604 U/mL and Dextrozyme DX 1.5X (Novozymes, Denmark) consisted of glucoamylase with an enzyme activity of 217.95 AGU/mL, were used for the hydrolysis of food waste. One unit of α-amylase is defined as the amount of enzyme that required to produce 1 mg of maltose from starch in 3 min at pH

6.9, 20°C. One unit of glucoamylase is defined as the amount of enzyme that cleaves 1 µmol of maltose per min at pH 5.0, 37°C.

Oleaginous yeast. Oleaginous yeast, *Rhodotorula glutinis* TISTR 5159, *Yarrowia lipolytica* TISTR 5621 and *Rhodosporidium toruloide*s TISTR 5123, were obtained from Thailand Institute of Scientific and Technological Research (TISTR). The oleaginous yeasts were maintained on yeast extract-malt extract (YM) agar slant (20 g/L agar, 10 g/L glucose, 5 g/L peptone, 3 g/L yeast extract and 3 g/L malt extract) at 4 °C and sub-cultured every month. The pre-culture was prepared by transferring one loop full of cultured yeast into YM media. The culture was conducted in shaking incubator (Thermostable IS-20R, Korea) at 30 °C with 160 for 24 h.

Food waste preparation

The lipid-free food waste preparation was modified from Yang et al. (2014). Dried food waste was extracted twice with hexane at ratio of 1:2 (w/v) and stirred at room temperature (30 ± 1°C) for 30 min. After that, the mixture was filtered and solid residue was dried in hot air oven at 60 °C. The lipid-free food waste was used as raw material for enzymatic hydrolysis of food waste.

Hydrolysis of food waste

Enzymatic hydrolysis of lipid-free food waste was performed according to the modified method of Yan et al. (2011); glucoamylase loading of 142.2 U/g of food waste, α-amylase loading of 10 U/g of food waste. Two enzyme solutions were simultaneously added to 30% (w/v) food waste and then incubated at 55 °C, pH 4.82 with 150 rpm in shaking incubator for 2 h. Then, the mixture was boiled for 10 min and was then centrifuged (Hettich Rotanta 46R, Germany) (4,000 rpm, 4 °C) for 15 min. Food waste hydrolysate (liquid phase) was collected for reducing sugar determination by DNS method.

Oleaginous yeast cultivation and lipid production

The food waste hydrolysate as culture medium was prepared by dilution into initial reducing sugar concentration at 20 g/L with reverse osmosis (RO) water. The oleaginous yeast cultivation and lipid production on food waste hydrolysate was conducted in 250 mL Erlenmeyer flask containing 50 mL of food waste hydrolysate sterilized at 121 °C for 15 min. Pre-culture (10%, v/v) was inoculated to food waste hydrolysate. The cultivation condition was performed in shaking incubator at 30 °C, 160 rpm for 384 h. The sample was harvested at 48 h intervals for remaining reducing sugar concentration, biomass

and lipid content determinations. The experiments were carried out with triplicate.

Biomass determination

The biomass or dry cell weight was determined gravimetrically. The culture broth was centrifuged with 3,500 rpm, at 4 °C for 10 min. Cell pellet was washed with RO water twice and dried by freeze-drying until constant weight.

Lipid extraction and determination

The lipid extraction method was modified from Meullemiestre et al. (2016). The process was carried out in screw cap glass tube containing 6.70 g glass beads (diameter of 3 mm) and 0.1 g dried cell pellets. Solvent mixture of chloroform and methanol (2:1, v/v) was added and stirred at room temperature for 30 min with vortex. The mixture was centrifuged at 3,500 rpm for 5 min. The lower phase was collected and evaporated for lipid determination by gravimetrical method.

Fatty acid methyl ester preparation

Fatty acid methyl ester (FAME) was prepared by acid-catalyzed transesterification which applied from Yang et al. (2014). 5% (v/v) H_2SO_4 in methanol and lipid sample were mixed in molar ratio of 6:1. The reaction was conducted at 80 °C with constant stirring for 3 h. The reaction mixture was cooled, and then added 10 mL of hexane. Upper layer containing FAME was collected for fatty acid composition analysis by gas chromatography/mass spectrometry (GC/MS).

Analytical methods

Sugar analysis. The reducing sugar was determined by using DNS method (Miller, 1959). The sugar composition was analyzed by high performance liquid chromatography (HPLC) HP 1100 series (Agilent, United States) equipped with Alltech Econsphere NH_2 (5 µm) 250 × 4.6 mm column and refractive index (RI) detector. The hydrolysate was filtered through 0.22 µm nylon membrane filter (Whatman) and acetonitrile (90%) was used as mobile phase at flow rate of 1 mL/min. D-glucose, sucrose, fructose, maltose, lactose, arabinose and xylose were used as standard sugars.

Fatty acid composition analysis. The FAME was performed with gas chromatograph/mass spectrometry HP 6890 Series (Agilent, United States)

coupled with HP-5ms column (30 m length × 0.25 µm film) and HP 5973 mass selective detector (MSD). Sample (1 µL) was injected at temperature of 220 °C with split ratio of 1:100. The initial temperature was set at 60 °C, and then increased to 260 °C at a rate of 5 °C/min and held for 3 min. Helium was used as carrier gas at a flow rate of 1 mL/min. The fatty acid composition was identified based on the comparison with database of Wiley 275 library of mass spectra.

Statistical analysis. The statistical analysis of results were evaluated using Statistix version 8.0 software. Tukey's HSD (honestly significant difference) was used to compare means at the 5% significance level.

RESULTS

Chemical composition of food waste

The chemical composition of food waste are presented in Table 1. The total dried solid of food waste (90.46 ± 0.53%, w/w) was contained with carbohydrate as the major component (49.24 ± 1.12%, w/w). The fiber content of 0.40 ± 0.08% (w/w) was the lowest component. The protein and lipid content were 17.53 ± 0.35% (w/w) and 18.09 ± 0.96%, (w/w), respectively.

Table 1. Chemical composition of canteen food waste.

Composition	Content (% dry basis, w/w)
Total solid	90.46 ± 0.53
Moisture	9.54 ± 0.53
Carbohydrate	49.24 ± 1.12
Lipid	18.09 ± 0.96
Protein	17.53 ± 0.35
Ash	5.19 ± 0.28
Fiber	0.40 ± 0.08

Results are presented as means ± S.D.

Enzymatic hydrolysis of food waste

The total reducing sugar concentration of 169.13 ± 5.67 g/L was obtained from enzymatic hydrolysis of 30% (w/v) lipid-free food waste. The HPLC chromatogram of the mixture standard sugar including xylose, arabinose, fructose, glucose, sucrose, maltose and lactose showed signal peaks

at retention time of 6.134, 6.783, 7.553, 8.385, 13.665, 15.396 and 18.059 min, while the food waste hydrolysate presented a retention time of 7.566, 8.349, 13.536 and 15.170 min as shown in Figure 1A. The results revealed that food waste hydrolysate was composed of glucose, fructose, maltose and sucrose. Glucose was the highest content of 77.83 ± 3.03% of total sugar. Sucrose and maltose were found at 15.30 ± 1.81 and 4.39 ± 0.72% of total sugar, respectively. The lowest sugar component was fructose (2.47 ± 0.26%, w/v) (Figure 1B).
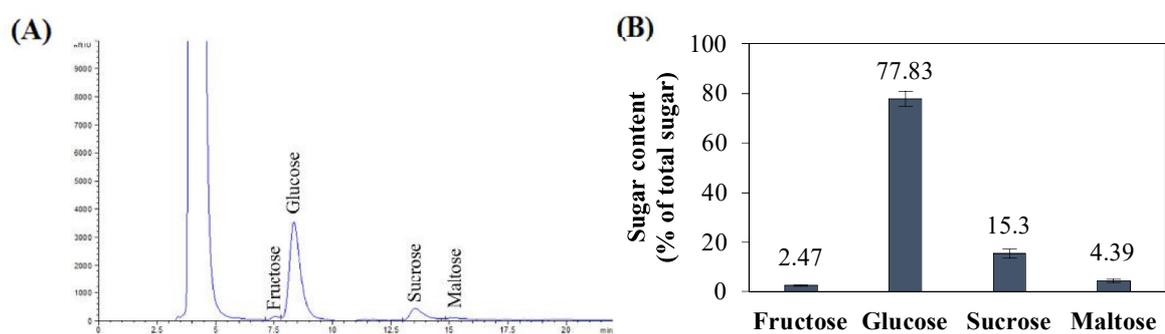

Figure 1. HPLC chromatogram of sugars (A) and sugar composition (B) of food waste hydrolysate from enzymatic hydrolysis of 30% (w/v) solid loading of food waste.

Oleaginous yeast cultivation and lipid production on food waste hydrolysate

The growth and lipid production of oleaginous yeasts (*R. glutinis* TISTR 5159, *Y. lipolytica* TISTR 5621 and *R. toruloides* TISTR 5123) on food waste hydrolysate is shown in Figure 2. Figure 2A presented that the highest biomass of *R. glutinis* TISTR 5159 (5.91 ± 0.15 g/L) and *Y. lipolytica* TISTR 5621 (3.95 ± 0.06 g/L) were found at 384 h and 96 h of the cultivation, respectively. The lipid content is presented in Figure 2B. The highest lipid content of *R. glutinis* TISTR 5159 (11.01 ± 1.20 % of dry biomass) and *Y. lipolytica* TISTR 5621 (12.82 ± 1.20 % of dry biomass) were found at 288 h and 96 h, respectively. The alteration of lipid concentration was related to the biomass and lipid content (Figure 2C). The lipid concentration of *R. glutinis* TISTR 5159 was gradually increased to the highest value of 0.61 ± 0.10 g/L at 384 h. The highest lipid concentration of *Y. lipolytica* TISTR 5621 was 0.51 ± 0.05 g/L which was found at 96 h. The remaining reducing sugar concentration (Figure 2D) was observed that the decreasing of reducing sugar concentration

were occurred during *R. glutinis* TISTR 5159 and *Y. lipolytica* TISTR 5621 cultivation. The reducing sugar concentration of *R. glutinis* TISTR 5159 was gradually decreased to the lowest value (4.33 ± 0.60 g/L) at 384 h. While the decreasing of reducing sugar concentration of *Y. lipolytica* TISTR 5621 cultivation was rapidly decreased to 3.28 ± 0.03 g/L within 96 h. Nevertheless the lipid production and the decreasing of reducing sugar concentration from *R. toruloides* TISTR 5123 cultivation were not appeared. The total biomass was standing in constant value approximately of 0.23 ± 0.02 g/L during 384 h of cultivation.
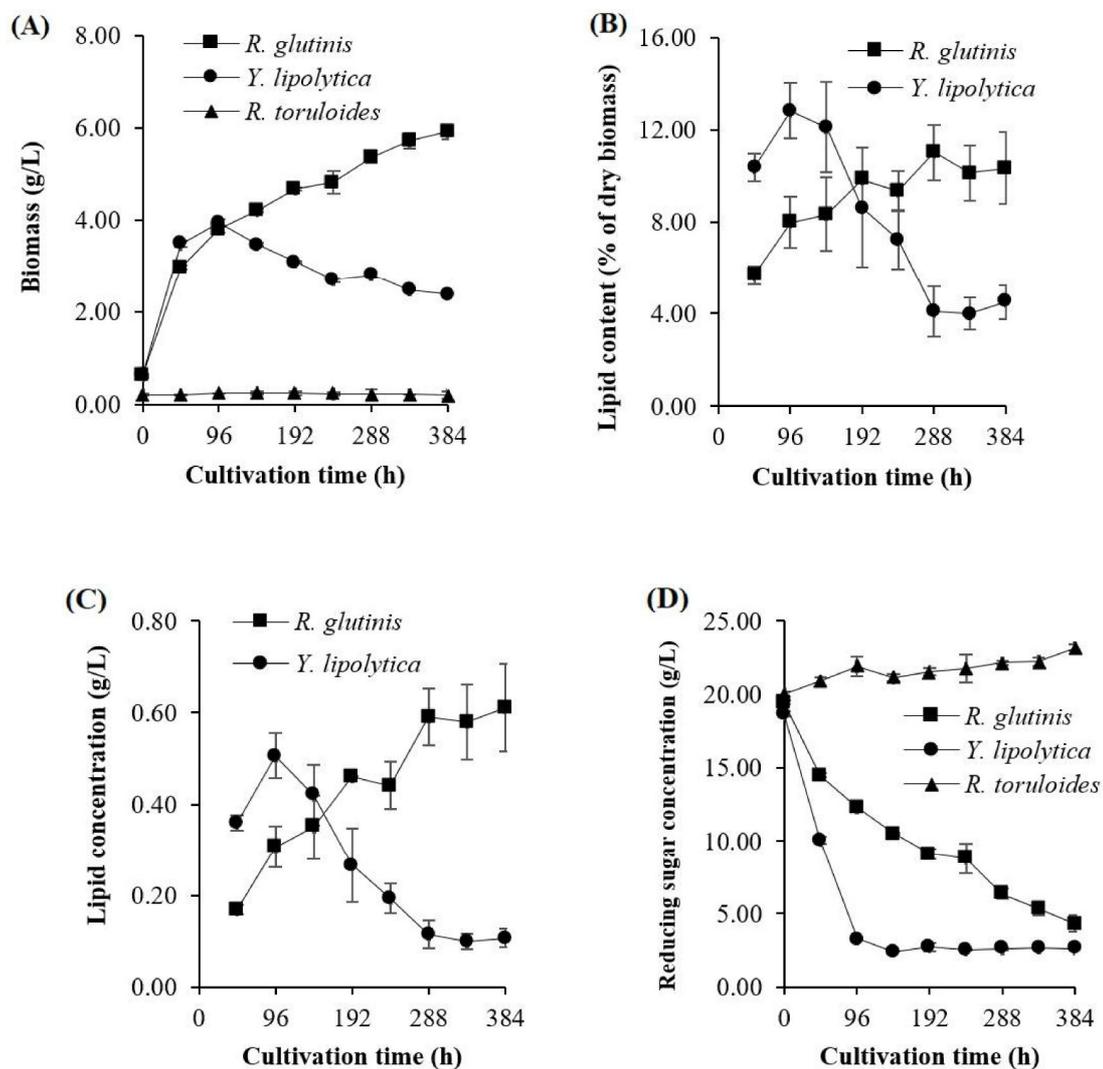
Figure 2. Growth and lipid production of oleaginous yeasts on food waste hydrolysate; biomass (A); lipid content (B); lipid concentration (C); remaining reducing sugar concentration (D).

Fatty acid composition

GC chromatogram of fatty acid and fatty acid content of *Y. lipolytica* TISTR 5621 lipid are presented in Figure 3A and 3B, respectively. The result demonstrated that the lipid was consisted of C16 and C18 chain length. The main fatty acid component was C18 (75.82%) which consisted of C18:0, C18:1 and C18:2, followed by C16 (24.61%) containing C16:0 and C16:1. The other fatty acid was found in a trace amount.
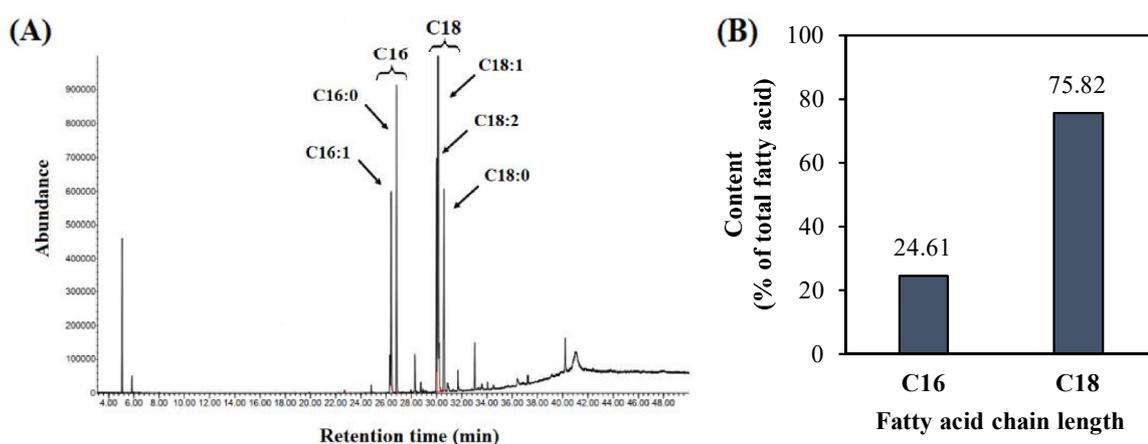
Figure 3. GC chromatogram of fatty acid (A) and fatty acid composition (B) of lipid derived from *Y. lipolytica* TISTR 5621.

DISCUSSION

The characteristic of canteen food waste was contained with rice, noodle, meat, pork and vegetable. The highest carbohydrate with the lowest fiber content indicated that starch and some sugars residue were contained in high proportion. This result was according to Moon et al., (2009); Yan et al., (2011); Hafid et al., (2015) that reported carbohydrate as the major constituent of food waste with the content of 47.70 to 63.87%. The food waste rich in carbohydrate, especially starch, is the potential raw material for fermentable sugar production via amylolytic enzyme hydrolysis. The separation of lipid from food waste was carried out prior the enzymatic hydrolysis in order to prevent the interfering of lipid residue contained in food waste hydrolysate in microbial lipid production. However, the extracted lipid from food waste have been reported as feedstock for biodiesel production with conversion efficiency of 89.8 to 100% (Yang et al., 2014; Karmee et al., 2015).

The enzymatic hydrolysis indicated that one step hydrolysis by α-amylase and glucoamylase had effectively degraded lipid-free food waste containing high carbohydrate content, especially starch. Food waste hydrolysate was contained with the major content of glucose and small amount of fructose and maltose. The low maltose content after hydrolysis indicated that maltose was almost completely hydrolyzed. The sucrose content (15.30%, w/v) was quite high which might be due to the addition of such sugar in cooking and seasoning step. However, sucrose could be supported the growth according to the expression invertase activity of oleaginous yeasts (Nicaud et al., 1989; Rubio et al., 2002). Therefore, food waste hydrolysate could be used as the alternative carbon source for oleaginous yeast cultivation.

The cultivation of oleaginous yeast demonstrated that *R. glutinis* TISTR 5159 and *Y. lipolytica* TISTR 5621 can be grown and produced lipid on food waste hydrolysate as observed by the increasing of total biomass, lipid content and lipid concentration. In meanwhile, the reducing sugar concentration of culture medium from oleaginous yeast cultivation was also decreased. However, the lipid productivity of *Y. lipolytica* TISTR 5621 was preferable because of the high lipid content and concentration were found within 96 h of cultivation. For *R. toruloides* TISTR 5123 was found that food waste hydrolysate was not appropriate for growth and also lipid production. This might be due to the improper characteristic of medium or some unfavorable compounds contained in food waste hydrolysate that could be inhibited the growth. The by-products contained in food waste including aldehydes, vanillin, p-benzaldehyde and organic acids (lactic acid, formic acid, acetic, propionic acid, butyric acid, levulinic acid, etc.) have been reported as inhibitors on oleaginous yeast growth (Sitepu et al., 2014; Gao et al., 2017). Nevertheless, Ma et al. (2018) revealed that *R. toruloides* 2.1389 could be produced lipid from lipid-free canteen food waste hydrolysate. The total biomass and lipid concentration were 12.09 g/L and 6.37 g/L, respectively. This might be depending on oleaginous yeast strain and type of food waste. *R. toruloides* 2.1389 have been reported that it has high tolerance on various organic waste substrates such as acetic acid, distillery wastewater and food waste hydrolysate (Huang et al., 2016; Ling et al., 2013; Ma et al., 2018).

The results can be concluded that food waste hydrolysate can be able to use for some oleaginous yeast cultivation and lipid production. For higher lipid production, the proper reducing sugar concentration of food waste or the improvement of culture medium constituent including supplemented with external carbon source or nutrients to increase C/N ratio need to investigate (Chi et al., 2011). According to Liu et al. (2015) who found that the high lipid

content (36.4%) and lipid concentration (5.5 g/L) were achieved from *R. glutinis* cultivation on undetoxified corncob hydrolysate with C/N ratio of 75. Additionally, the highest lipid concentration of 9.15 g/L and lipid content of 60.0% were achieved by *R. glutinis* TISTR 5159 cultivation on pineapple pulp hydrolysate with C/N ratio of 77 (Tinoi and Rakariyatham, 2016). For *Y. lipolytica* SKY7 cultivation and lipid production, Kuttiraja et al. (2016) presented that the maximum lipid content was found to be 44.6% on glycerol with C/N ratio of 100 as substrate. Moreover, inhibitory compounds contained in food waste hydrolysate should also be analyzed.

The fatty acid composition of lipid produced from *Y. lipolytica* TISTR 5621 was mainly consisted of C16 and C18 chain length approximately 99% of total fatty acid. This was comparable to Andre´ et al. (2009); Makri et al. (2010); Canonico et al. (2016) who revealed that fatty acid component of lipid from *Y. lipolytica* cultivation on glycerol were C16 and C18 in range of 95 to 99%. The main fatty acid composition with C16 and C18 of oleaginous yeast lipid is similar to plant oil or animal fat (Beopoulos et al., 2009). This could be made the lipid of *Y. lipolytica* TISTR 5621 as raw material for value-added products production such as dietary supplements, cocoa butter substitutes, biodiesel, etc. (Meng et al., 2009; Papanikolaou and Aggelis, 2011; Chuan-chao et al., 2007; Li et al., 2008).

CONCLUSION

The canteen food waste was mainly consisted of carbohydrate. The amylolytic enzyme hydrolysis of lipid-free food waste provided the reducing sugar concentration of 169.13 g/L with high glucose content (77.83% of total sugar). Food waste hydrolysate was preliminary utilized on growth and lipid production of oleaginous yeast. The results found that food waste hydrolysate was a promising culture medium for *R. glutinis* TISTR 5159 and *Y. lipolytica* TISTR 5621. *Y. lipolytica* TISTR 5621 was the efficiency oleaginous yeast with gave high lipid productivity and achieved the highest lipid content and lipid concentration within 96 h of the cultivation. The lipid of *Y. lipolytica* TISTR 5621 was mainly consisted of C16 and C18 fatty acid component as high as 99% of total fatty acid. This result indicated that food waste hydrolysate could be used as an alternative feedstock for oleaginous yeast cultivation and lipid production.

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