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Research article

Enabling cassava starch hydrolysate for *Pseudozyma tsukubaensis* YWT 7-2 oil production using $Ca(OH)_2$ treatment

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Abstract

Cassava starch hydrolysate (CSH) was evaluated as feedstock for *Pseudozyma tsukubaensis* YWT 7-2 oil production using a two-step cultivation method. *P. tsukubaensis* YWT 7-2 produced oil in the CSH (without supplementation) only after the CSH was treated with $Ca(OH)_2$ at pH 10 (CaCSH). Addition of 0.1% (weight per volume, w/v) KH_2PO_4 into the CaCSH containing 0.2% (w/v) $(NH_4)_2SO_4$ increased the resultant cell mass 1.49-fold in the cell propagation step, while supplementation of the CaCSH with 0.25% (w/v) $(NH_4)_2SO_4$ increased the oil titer 2.52-fold in the oil production step. The optimal pH and cell inoculum for oil production were 5.5 and 8.05 g (dry cell weight)/L, respectively. The major fatty acids in the oil from *P. tsukubaensis* YWT 7-2 grown in CaCSH were palmitic (47.8%) and oleic (36.96%) acids, somewhat similar to those of palm oil and so indicating their potential use as feedstock for biodiesel production. The results showed that the $Ca(OH)_2$ treatment of CSH, which is high in sugar but low in nitrogen, was indispensable for *P. tsukubaensis* YWT 7-2 oil production.

Introduction

Yeasts that accumulate oil to more than 20% (weight per weight dry cell weight; DCW) are defined as oleaginous yeasts (Ratledge, 1989). Under excess carbon but limited nitrogen culture conditions, when the nitrogen source is exhausted, the oleaginous yeasts, which have adenosine monophosphate (AMP) deaminase and ATP-citrate lyase, synthesize lipid from the remaining carbon source and intracellularly accumulate the lipid as an oil droplet (Qin et al., 2017). The above condition activates AMP deaminase to liberate ammonia (as ammonium ions; NH_4^+) from AMP by changing AMP to inosine monophosphate and NH_4^+. The excessive decrease in the AMP concentration inactivates isocitrate dehydrogenase, an AMP-allosteric enzyme that catalyzes isocitrate to -ketoglutaric acid in the Krebs cycle, and results in the accumulation of isocitrate in the mitochondria. Isocitrate and citrate are maintained in equilibrium with each other in the mitochondria

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by the reversible activity of isocitrate acotinase. When the concentration of citrate in the mitochondria reaches a critical value, the citrate enters the cytoplasm in exchange with malate, and then the citrate is cleaved by ATP-CL into acetyl-CoA and oxaloacetate. The acetyl-CoA is catalytically converted by acetyl-CoA carboxylase into malonyl-CoA (Papanikolaou and Aggelis, 2011), which is catalyzed through a series of reactions by fatty acid synthase to become long chain acyl-CoAs derivatives. These long chain acyl-CoA derivatives are channeled into the biosynthetic pathway of cellular lipids, such as phospholipids and triacylglycerol (TAG) (Fakas, 2017). Most of the accumulated oil was found in the form of TAG (Poontawee et al., 2017).

The main fatty acids found in yeast oils are myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic, (C18:2) and linolenic (C18:3) acids (Patel et al., 2016). However, different oleaginous yeast species have different fatty acid compositions, and the fatty acid profile is totally dependent on the culture medium and conditions (Meng et al., 2009; Tanimura et al., 2014). For example, factors reported to affect yeast oil accumulation included the carbon and nitrogen resources, the carbon-nitrogen (C/N) ratio, temperature, pH, oxygen, concentration of trace metal ions and inorganic salts (Qiang et al., 2008).

Typically, yeast oils have fatty acid profiles similar to some plant oils that are used as feedstock for biodiesel production (Ageitos et al., 2011), which indicates the potential of yeast oil as alternative conventional biodiesel feedstock. The advantages of yeast oil over plant oil production are its short life cycle, ease of culturing and up-scaling, lower land use requirement, independence of climatic conditions and the ability to utilize various carbon sources (Juanssilfero et al., 2018).

Almost 30 million t of cassava are generated each year in Thailand, which contain 71.9–85.0% (weight per weight, w/w) starch and only 1.57–5.78% (w/w) protein, according to The Thai Tapioca Development Institute (2019). The high availability and the high C/N ratio of cassava make cassava starch of interest as a feedstock for yeast oil production. Interestingly, cassava starch hydrolysate (CSH) has been used as carbon source for oil production from *Rhodosporidium toruloides* 21167 (Wang et al., 2012) and *Rhodotorula mucilaginosa* TJY15a (Li et al., 2010).

Recently, an oleaginous yeast, *Pseudozyma tsukubaensis* YWT 7-2, was isolated from a mangrove forest located in Chanthaburi province, Thailand. This was the first time an isolate of *P. tsukubaensis* has been shown to be oleaginous. As the fatty acid composition of oleaginous yeast oils is species specific, the objective of the current study was to evaluate the possibility of using CSH as feedstock for production of *P. tsukubaensis* YWT 7-2 oil and to determine the application potential of the oil produced.

Materials and Methods

Microorganism and enzymes

The oleaginous yeast, *P. tsukubaensis* YWT 7-2 (preserved in a metabolically inactive state in the culture collection at the Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand under accession number MSCU 1163), α-amylase (120,000 units (U)/mL) and glucoamylase (220,000 U/mL) (Siam Victory Chemicals, Co. Ltd., Thailand) were used in this study.

Preparation of cassava starch hydrolysate and $Ca(OH)_2$-treated cassava starch hydrolysate

The CSH containing 10% (weight per volume, w/v) glucose was prepared by liquefying 20% (w/v) cassava starch with α-amylase (26.4 U/g) at 85°C and pH 5.8 for 4 hr and then further saccharifying it using glucoamylase (16.5 U/g) at 60°C and pH 4.5 for 2 hr. After centrifugation at 9,803×*g* for 20 min, the resultant clear syrup was adjusted to pH 5.5 and filtered through Whatman No. 1 filter paper. The filtrate was referred to as CSH.

The lipid content of *Rhodosporidium kratochvilovae* HIMPA1 grown under both nitrogen and phosphorus limited conditions was higher than when grown in either a nitrogen or a phosphorus limited condition (Patel et al., 2017). Since soluble phosphate can be removed via precipitation with Ca^{2+} (Wu et al., 2010), to examine the oil production of *P. tsukubaensis* YWT 7-2 under both nitrogen and phosphorus limited conditions, CSH limited in both nitrogen and phosphorus was prepared using $Ca(OH)_2$ treatment (Yu et al., 2011) by adding $Ca(OH)_2$ into the CSH until the pH reached 10, and stirring continuously at 50°C for 20 min. Then, it was centrifuged, the supernatant was harvested, adjusted to pH 5.5 and filtered as above. The CSH and CaCSH were sterilized by autoclaving at 110°C for 10 min. The concentrations of glucose, total nitrogen, total phosphorus and some ions in the CSH and CaCSH were analyzed as follows. The glucose concentration was analyzed using a biochemistry analyzer (YSI 2700 Select, YSI Incorporated; Yellow Springs, OH, USA). The total nitrogen content was determined using the Kjeldahl method, total phosphorus by

the ascorbic acid method and the levels of iron, manganese, copper, zinc, calcium, magnesium and potassium ions using atomic absorption spectrometry at the Environmental Research Institute, Chulalongkorn University, Bangkok, Thailand. The C/N ratio was calculated using Equation 1:

$$C/N = [(glu) \times 6 / 180[/ ([N] / 14) \quad (1)$$

where [glu] and [N] are the initial glucose and the total nitrogen concentrations, respectively.

P. tsukubaensis YWT 7-2 oil production

Oil was produced by *P. tsukubaensis* YWT 7-2 in a two-step cultivation process due to the different nutrient requirements of cells in the growth and oil production phases. In the first step, cell propagation, *P. tsukubaensis* YWT 7-2 cells were grown in yeast and mold (YM) medium (glucose 1%, yeast extract 0.3%, malt extract 0.3%, peptone 0.5%, all (w/v), pH 5.5) at room temperature (32°C) for 48 hr with agitation at 200 rpm. Then, the resultant culture (100 mL) was centrifuged (4°C, 9,803×*g*, 10 min) and the cells were washed with oil production medium (OPM). In the second step, oil production, the washed cells were inoculated into 100 mL of OPM and incubated at room temperature (32°C) and 200 rpm for 4 d. Every day, the culture was harvested using centrifugation and the obtained cells were washed with distilled water, dried by lyophilization and weighed. The weight of the lyophilized cells was recorded as the dry cell weight (DCW) of the obtained cell mass. Synthetic high C/N medium [glucose 5%, $(NH_4)_2SO_4$ 0.1%, yeast extract 0.1%, $MgSO_4 \bullet 7H_2O$ 0.005%, KH_2PO_4 0.1%, $CaCl_2 \bullet 2H_2O$ 0.001%, NaCl 0.001%, all (w/v), pH 5.5], 5% (w/v) glucose, CSH or CaCSH was used as the OPM as indicated.

Maximization of P. tsukubaensis YWT 7-2 oil production in cassava starch hydrolysate

Increase of the *P. tsukubaensis* YWT 7-2 oil production in CSH was done by examining the necessity of growth essential nutrients in oil production medium by comparison of the oil production in the synthetic high C/N medium and glucose solution. Next, as simultaneous limitation of nitrogen and phosphorus condition of oil production medium was reported to increase oil production of *Rhodosporidium kratochvilovae* (Patel et al., 2017), oil production of the *P. tsukubaensis* YWT 7-2 in CaCSH, from which soluble phosphate was removed by precipitation with Ca^{2+}, was compared with CSH. Then, optimal C/N ratio and initial pH of the selected oil production medium including inoculum size were determined.

Analysis of intracellular oil accumulated

Oil accumulated in the lyophilized cells was extracted as reported by Pranimit et al. (2019). In brief, the lyophilized cells (0.5 g) were suspended in 10 mL of 2:1 (v/v) chloroform:methanol and sonicated at 37 kHz for 15 min. After adding 0.73% (w/v) NaCl to make a 2:1:0.8 (volume per volume per volume) chloroform:methanol:water mixture, it was centrifuged (4°C, 540×*g*, 10 min) to separate the oil-containing lower phase. The residual solvent in the harvested oil phase was removed by evaporation at room temperature and the resultant dried oil residue was weighed. The oil titer (measured in grams per liter) was calculated as the amount of oil extracted from the cells per liter broth and oil content (based on %, w/w) was defined as the percentage of oil titer (in grams per liter) relative to the dry cell weight (in grams per liter). The oil yield (grams per gram, g/g) refers to the percentage of oil titer (in grams per liter) relative to the sugar consumption (in grams per liter).

Analysis of fatty acid composition

The oil was extracted, transesterified and analyzed as reported by Pranimit et al. (2019). In brief, cells (1 g) were suspended in 0.8 mL of 10% (w/v) potassium hydroxide in methanol and incubated at 80°C for 2 hr. After cooling to room temperature, they were extracted with petroleum ether (1 mL) and centrifuged (4°C, 9,803×*g*, 10 min). The resultant aqueous phase was harvested, acidified by the addition of 3 mL of 6 N hydrochloric acid, and extracted with diethyl ether. Fatty acids were recovered from the diethyl ether phase by evaporation using nitrogen gas and transmethylated with boron trifluoride. The fatty acid methyl esters obtained were extracted with hexane and analyzed using gas chromatography (GC; 7890 B, Agilent Technologies; Santa Clara, CA, USA). The conditions used were: capillary column (0.32 mm × 30 m, 0.25 µm thickness), flame ionization detector at 150°C, injection and detection at 250°C, with helium as the carrier gas at a flow rate of 2.3 mL/min. The initial temperature was 150°C and then sequentially increased to 180°C at 10°C/min, to 200°C at 5°C/min, to 205°C at 0.5°C/min, then held for 2 min and finally increased to 250°C at 5°C/min and held for 5 min.

Statistical analysis

All data values are means ± standard deviation of three separate experiments. Difference in means were analysed using ANOVA for independent measures, followed by Duncan's multiple range test or independent samples t-test comparisons (SPSS version 22, IBM, Chicago, IL, USA). Differences were considered statistically significant for $p < 0.05$.

Results and Discussion

Comparison of oil production in synthetic high C/N medium or glucose solution

Cells of *P. tsukubaensis* YWT 7-2 grown in YM medium were used to produce oil in a synthetic high C/N medium or in 5% (w/v) glucose solution. In the synthetic high C/N medium, the highest cell mass and oil content were 14.65 g/L and 19.51% (w/w, DCW), while in the glucose solution they were 9.85 g/L and 18.94% (w/w, DCW), respectively. A decrease in the glucose concentration was observed in both the synthetic high C/N medium and glucose solution. This revealed that glucose was assimilated and converted into cell mass and accumulated oil. The maximum oil titer in the synthetic high C/N medium was 2.72 g/L (oil productivity of 0.91 g/L/d), while in the glucose solution it was 1.71 g/L (oil productivity of 0.57 g/L/d), as shown in Fig. 1. The results indicated that cell growth and oil accumulation of the *P. tsukubaensis* YWT 7-2 could not be temporarily separated. Thus, the OPM must contain a limited concentration of essential nutrients for growth and oil production. The lipid biosynthesis of *Lipomyces starkeyi* AS 2.1560 was reported to remain active for some time in the absence of essential nutrients for growth, where in a 4% (w/v) glucose solution, the cell mass of *L. starkeyi* AS 2.1560 increased less than 10%, while the oil content reached 58.6% (w/w, DCW) (Lin et al., 2011).

Oil production in cassava starch hydrolysate or calcium cassava starch hydrolysate media

For the *P. tsukubaensis* YWT 7-2 oil production in CSH or CaCSH media with or without supplementation, the cells were first grown in CSH medium supplemented with 0.2% (w/v) $(NH_4)_2SO_4$ (C/N ratio of 106) at pH 5.5. In the CSH, the cell mass of the *P. tsukubaensis* YWT 7-2 gradually decreased after inoculation and resulted in no oil production. In the CaCSH,

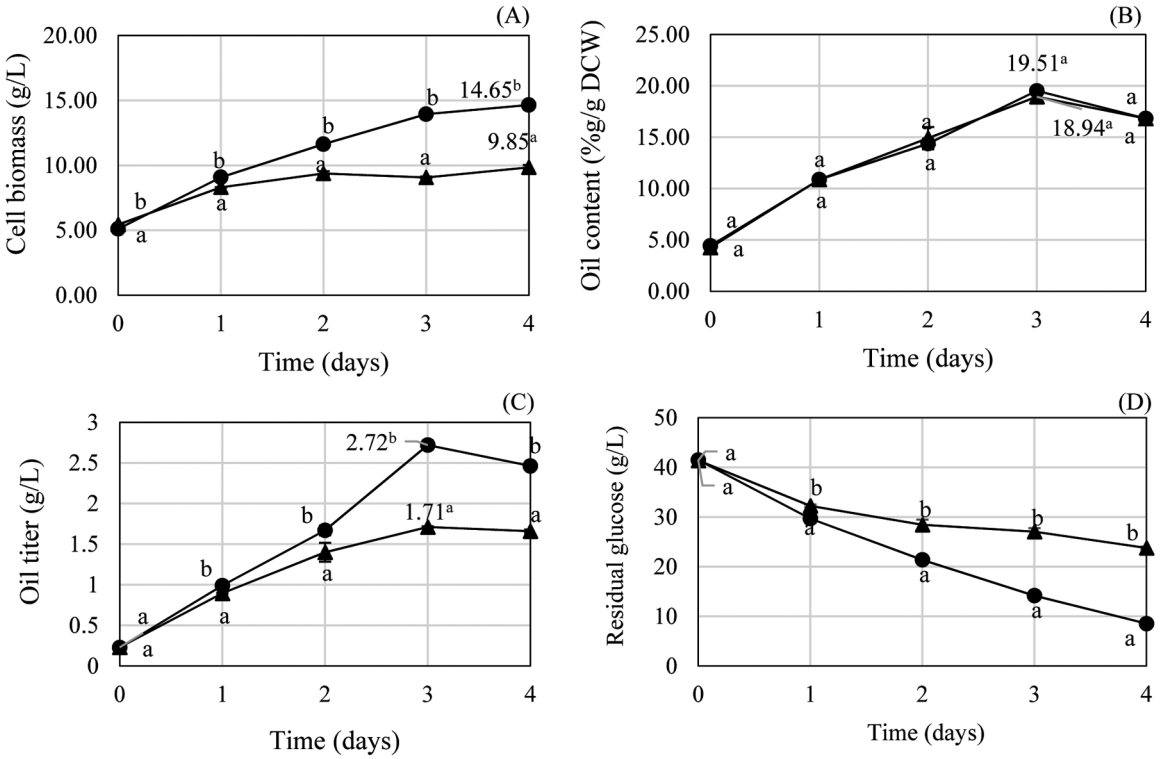

Fig. 1 Comparison of: (A) cell mass; (B) oil content; (C) oil titer; (D) residual glucose of *Pseudozyma tsukubaensis* YWT7-2 grown in synthetic high C/N medium (●) or 5% (w/v) glucose solution (▲). DCW = dry cell weight

the maximum oil content of 35.43% (w/w, DCW) was recorded at 4 d and the maximum cell mass (5.82 g/L) was at 1 d with a maximum oil titer of 1.49 g/L (oil productivity of 0.75 g/L/d) (Fig. 2). Without supplementation, *P. tsukubaensis* YWT 7-2 could grow in the CaCSH but not in the CSH. There was a slight decrease in the glucose concentration and no significant differences in the concentrations of ions reported to have effects on oil production of oleaginous yeasts (Ageitos et al., 2011) in the CaCSH. However, the phosphorus concentration in the CaCSH was reduced by 39.8% compared to CSH (Table 1). Phosphorus is essential for growth and lipid accumulation in oleaginous yeast cells as well as for the maintenance of various metabolic activities, such as the synthesis of DNA and phospholipids and the formation of co-enzymes (Patel et al., 2017). The treatment with $Ca(OH)_2$ has been reported to detoxify the acid hydrolysate by causing chemical conversion and not by precipitation of one or more inhibitors (Jönsson et al., 2013). In the same way, chemical conversion of the CaCSH by $Ca(OH)_2$ treatment might support the growth of *P. tsukubaensis* YWT 7-2 in the CaCSH. The CaCSH was selected as the medium for use in oil production in the next experiments.

Effect of $(NH_4)_2SO_4$ supplementation in calcium cassava starch hydrolysate medium on oil production

The oil accumulation in *P. tsukubaensis* YWT7-2 was sub-optimal in the absence of essential nutrients for growth (Fig. 1). Rather, a suitable C/N ratio was necessary for the

Table 1 Chemical composition of the cassava starch hydrolysate (CSH) and calcium CSH (CaCSH)

Composition	CSH	CaCSH
Glucose (g/L)	95.7 (mg/L)	93.5 (mg/L)
Total nitrogen[a]	ND	ND
Iron[b]	0.11	0.17
Manganese[b]	0.08	0.12
Copper[b]	< 0.10	< 0.10
Zinc[b]	0.14	0.35
Calcium[b]	7.81	137
Magnesium[b]	13.2	13.6
Potassium[b]	25.0	23.8
Total phosphorus[c]	2.69	1.62

ND = not detectable

Assayed using [a]Kjeldahl, [b]atomic absorption spectrometry and [c]ascorbic acid methods

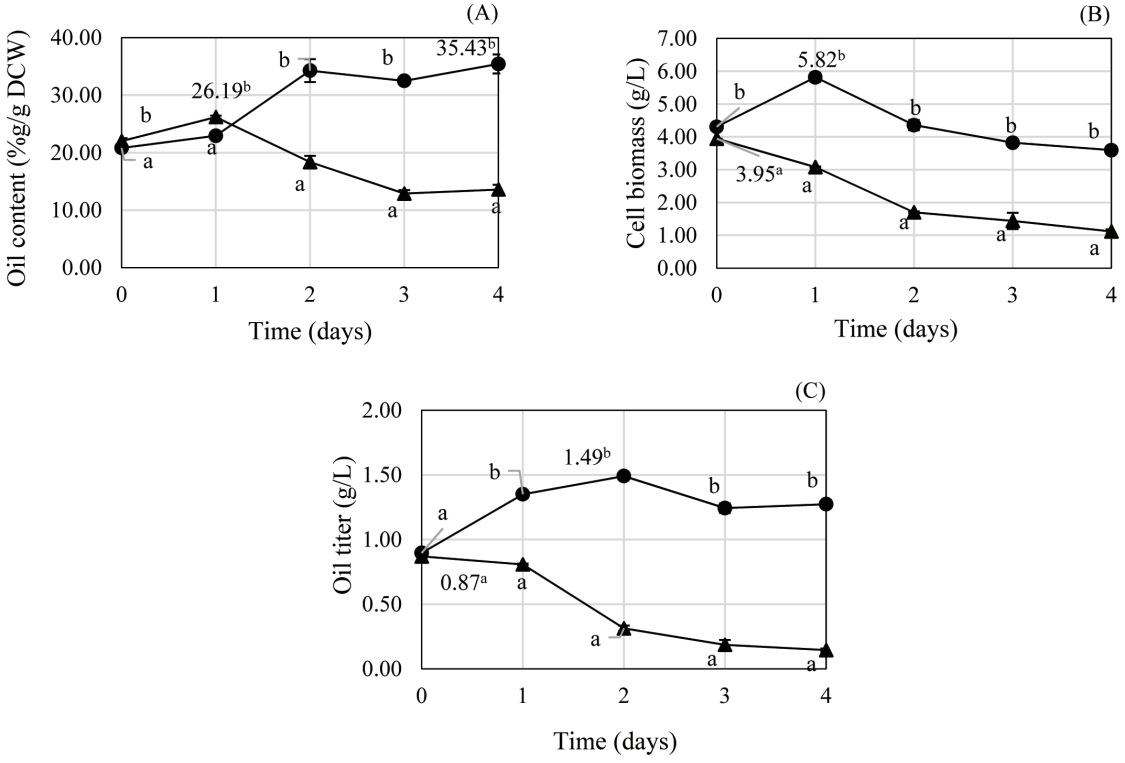

Fig. 2 Comparison of: (A) oil content; (B) cell mass; (C) oil titer of *P. tsukubaensis* YWT7-2 grown in cassava starch hydrolysate (CSH, ▲) or CaCSH (●) without supplementation. DCW = dry cell weight

growth and oil production of this oleaginous microorganism (Zhu et al., 2008). In this experiment, either 0.1%, 0.25% or 0.5% (w/v) $(NH_4)_2SO_4$ was added into the CaCSH to adjust the initial C/N ratio to 208, 82, and 41, respectively. It was found that supplementation of CaCSH with 0.25% (w/v) $(NH_4)_2SO_4$ produced the highest oil content in *P. tsukubaensis* YWT 7-2 of 31.87% (w/w, DCW) after 3 d and the highest cell mass of 12.7 g/L after 4 d. The maximum oil titer increased to 3.76 g/L, equal to oil productivity of 1.25 g/L/d (Fig. 3). The addition of 0.5% (w/v) $(NH_4)_2SO_4$ increased the maximum oil titers of *Trichosporonoides spathulata* and *Kodamaea ohmeri* in the crude glycerol to 4.13 g/L and 3.17 g/L, respectively (Kitcha and Cheirsilp, 2011). In addition, the maximum oil titer of *Rhodotorula glutinis* was obtained when the initial C/N ratio of corncob hydrolysate reached 75 by the addition of $(NH_4)_2SO_4$ (Liu et al., 2015).

The oil titer of *Trichosporon cutaneum* ACCC 20271 in corncob residue hydrolysate increased with an increasing concentration of added corn steep liquor (CSL) and reached a maximum (7.8 g/L) with 6 g/L CSL (C/N ratio of 49.3) (Gao et al., 2014). Batch cultivation of *Candida freyschussii* ATCC 18737 in medium containing 3 g/L yeast extract and various concentrations of glycerol in the range 4–160 g/L produced the highest oil titer of 4.6 g/L with 40 g/L glycerol (Raimondi et al., 2014). When the C/N ratio of pasteurized glucose-based medium containing 4% (w/v) NaCl was increased from 106 to 211, the oil titer of *R. toruloides* DSM 4444 increased from 5.9 g/L to 9.1 g/L (Tchakouteu et al., 2017). The maximum oil titer (1.93 g/L) of *Rhodotorula* sp. IIP-33 was achieved in sugarcane bagasse hydrolysate supplemented with 30 g/L xylose and 0.5 g/L $(NH_4)_2SO_4$ (Bandhu et al., 2014). *R. toruloides* DMKU3-TK 16 produced a maximum oil titer of 9.26 g/L when grown in an optimized nitrogen-limited medium II with a C/N ratio of 140 (Kraisintu et al., 2010).

Effect of initial pH on oil production

When *P. tsukubaensis* YWT7-2 was grown in CaCSH supplemented with 0.25% (w/v) $(NH_4)_2SO_4$ at various initial pH values (5.0, 5.5, 6.0), it was found that *P. tsukubaensis*

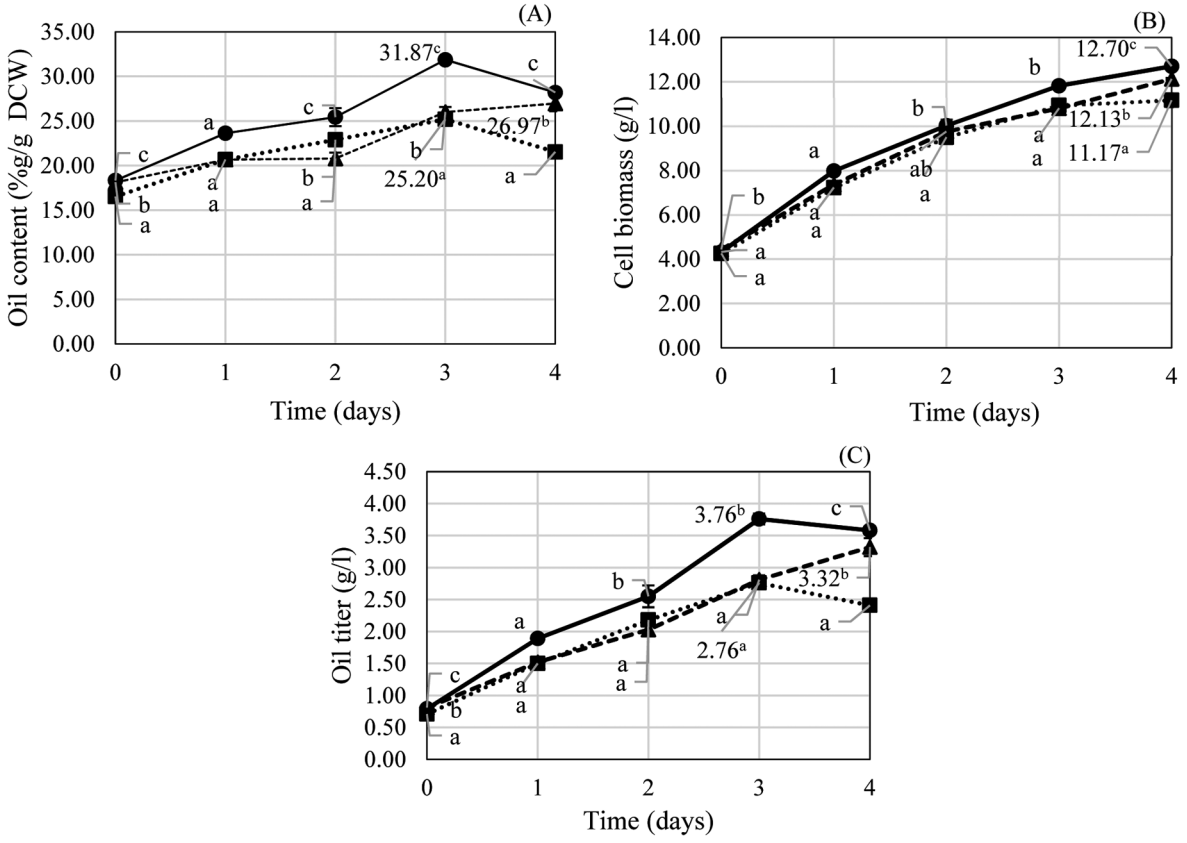

Fig. 3 Effect of $(NH_4)_2SO_4$ concentration supplemented in calcium cassava starch hydrolysate on oil production of *Pseudozyma tsukubaensis* YWT7-2: (A) oil content; (B) cell mass; (C) oil titer, when grown with 0.1% (■), 0.25% (●) or 0.5% (▲) weight per volume $(NH_4)_2SO_4$. DCW = dry cell weight

YWT7-2 had a maximum oil titer (4.45 g/L) at pH 5.0, equal to an oil productivity of 1.11 g/L/d. The highest oil productivity (1.25 g/L/d) was at pH 5.5, with an oil titer of 3.76 g/L (data not shown). Thus, CaCSH supplemented with 0.25% (w/v) $(NH_4)_2SO_4$, at an initial pH of 5.5 was used in the following experiments. The slightly acidic optimal culture medium (pH 5.5) for oil production noted in this study was consistent with another studies, where the initial pH of the culture medium had a substantial impact on the lipid productivity of the oleaginous yeast *Trichosporon fermentans* (Zhu et al., 2008). In addition, *Cryptococcus curvatus* NRRLY-1511 grown in beet molasses plus corn gluten produced a maximum amount of oil at pH 5.5 (El-Fadaly et al., 2009), while the optimal pH for oil production by *Rhodotorula kratochvilovae* SY89 in nitrogen-limited medium was also pH 5.5 (Jiru et al., 2017).

Effect of inoculum size on oil production

A suitable inoculum size can promote a higher oil yield (Kurosawa et al., 2010). Using the two-step cultivation method, the resultant cell biomass from the initial cell propagation step was used as the inoculum for the oil production step. For *P. tsukubaensis* YWT7-2 grown in CaCSH (without supplementation), as shown in Fig. 2, CaCSH supplemented with 2.0% (w/v) $(NH_4)_2SO_4$ was used as the first step cell propagation medium instead of CSH supplemented with 2.0% (w/v) $(NH_4)_2SO_4$. Just under 39.8% of the phosphorus, an element necessary for cell growth, was precipitated from the CaCSH during the $Ca(OH)_2$ treatment of CSH (Table 1); consequently, in this experiment, *P. tsukubaensis* YWT7-2 was grown in CaCSH supplemented with 0%, 0.1% or 0.2% (w/v) KH_2PO_4 (CaCSH-PM) and the CaCSH-PM culture that had the highest cell mass was selected as the inoculum to produce oil in the optimized OPM [CaCSH supplemented with 0.25% (w/v) $(NH_4)_2SO_4$, pH 5.5].

The highest *P. tsukubaensis* YWT7-2 cell mass (8.15 g DCW/L) was obtained in the CaCSH-PM supplemented with 0.1% (w/v) KH_2PO_4, followed by that with 0.2% and 0% (w/v) KH_2PO_4 at 7.20 g DCW/L and 5.39 g DCW/L, respectively (data not shown). When all the cells first grown in the CaCSH-PM supplemented with 0.1% (w/v) KH_2PO_4 were inoculated into the optimized OPM (inoculum size of 8.15 g DCW/L), the highest oil titer (5.20 g/L) was obtained, representing an oil productivity of 1.73 g/L/d and an oil yield of 24.8% g/g. Thus, the oil titer was enhanced by increasing the inoculum size, which was consistent with other work that reported an increased cell mass when using a higher inoculum size (Juanssilfero et al., 2018).

A Box-Behnken experimental design and response surface methodology were used to determine the effects of crude glycerol concentration (30–110 g/L), initial pH (3–7) and initial cell concentration (optical density (OD) at 600 nm of 15–56.5) on *Rhodosporidiobolus fluvialis* DMKU-RK253 oil production from crude glycerol using the two-stage cultivation process. The results revealed that the initial cell concentration had a significant effect on lipid production when a low concentration of glycerol was used. In contrast, there was no effect of the initial cell concentration on oil production when a high crude glycerol concentration was used. The optimal conditions for the oil production were 57 g/L crude glycerol, an initial pH of 7 and an initial concentration OD 20 at 600 nm in the second stage of cultivation (Polburee and Limtong, 2020). Apart from size, the type of inoculum also has an important role. Oil production of *R. toruloides* DSM 4444, a moderately halotolerant yeast, was possible under non-aseptic conditions by the addition of 4% (w/v) NaCl with only a 1.1% reduction in the oil titer (Tchakouteu et al., 2017).

Fatty acid composition of oil

The major fatty acids in the *P. tsukubaensis* YWT7-2 oil when grown in the synthetic high C/N medium were oleic (37.9%), palmitic (36.7%), linoleic (7.8%) and stearic (7.1%) acids (Fig. 4). In accord with this result, most yeast oils have palmitic, stearic, oleic and linoleic acids as the dominant fatty acids (Athenaki et al., 2017). The oil from *P. tsukubaensis* YWT7-2 produced in the CaCSH contained palmitic

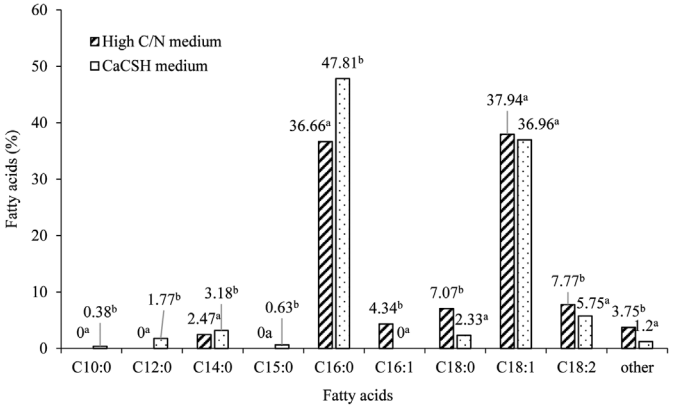

Fig. 4 Major fatty acids in oil of *Pseudozyma tsukubaensis* YWT7-2 grown in synthetic high C/N medium and calcium cassava starch hydrolysate (CaCSH) medium at optimized composition where acids are myristic (C14:0), palmitic (C16:0), palmitoleic (16:1), stearic (C18:0), oleic (C18:1) and linoleic (C18:2).

(47.8%), oleic (37%), linoleic (5.8%) and stearic (2.3%) acids (Fig. 4). The level of palmitic was 1.3-fold higher, while the oleic, linoleic and stearic acids were 1.1-, 1.3- and 3.1-folds lower, respectively, than the oil produced in the synthetic high C/N medium. Indeed, the feedstock composition has been reported to have a significant influence on the fatty acid profile of oleaginous oil (Brar et al., 2017).

The similar dominant fatty acids in *P. tsukubaensis* YWT7-2 oil as in palm oil indicates its potential use as a feedstock for biodiesel production (Ageitos et al., 2011). The dominant fatty acids in palm oil, a common plant oil feedstock for biodiesel production, were palmitic (44%), oleic (39.2%), linoleic (10.1%), and stearic (4.5%) acids (Mancini et al., 2015). A higher or almost equal saturated:unsaturated fatty acid ratio is preferred for lipids to qualify for use as a biodiesel feedstock (Chopra et al., 2018). However, as the *P. tsukubaensis* YWT7-2 oil had C16 and C18 fatty acids as the main fatty acids and an almost equal saturated:unsaturated fatty acid ratio, it might serve as a feedstock for cocoa butter substitutes production. Cocoa butter is extracted from cocoa beans and used in the chocolate fabrication and biscuit industries. It mainly contains three different kinds of TGAs containing (palmitic acid (P), oleic acid (O) and stearic acid (S) of the types P-O-P, P-O-S and S-O-S (Wei et al., 2017).

Oleic (51.6%), palmitic (21.6%), linoleic (17.7%) and stearic (5.8%) acids accounted for over 91% of the fatty acids found in the oil of *R. toruloides* 21167 co-cultured with *Saccharomycopsis fibuliger* A11-c, an amylase producing yeast, immobilized on polyvinyl alcohol in medium containing 6% (w/v) cassava starch (Gen et al., 2014). Likewise, these four fatty acids were dominant (96.8%) in the oil from *R. toruloides* 21167 grown in medium containing 8% (w/v) cassava starch (Wang et al., 2012). Major fatty acids of *R. toruloides* DSM 4444 oil produced during fed-batch cultivation using mixed confectionary waste hydrolysate were oleic (61.7%), palmitic (15.2%), stearic (13.8%) and linoleic (6.1%) acids (Tsakona et al., 2019). The oil from *R. mucilaginosa* TJY15a grown in medium containing 2% (w/v) cassava starch was mainly composed of oleic (63.5%), palmitic (22.3%), linoleic (5.7%) and stearic (5.2%) acids (Li et al., 2010). The current findings are useful as CSH, a high carbon low nitrogen feedstock abundant in Thailand, could produce a high-value yeast oil, such as palmitoleic acid and linoleic acid, providing optimum return on investment.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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