CHAPTER 4

Effect of additive agent on carotenoids and lipids productions by *Sporidiobolus pararoseus* KM281507

4.1 Introduction

Crude glycerol is a main by-product of biodiesel production process. As the biodiesel production are growing, the quantity of generated crude glycerol will be considering, as well as its utilization will become an urgent topic (Xu et al., 2012a). Moreover, crude glycerol has little value and disposal may be difficult because of its harmful to environment, while, purification of crude glycerol is industrially almost infeasible because of the high processing cost due to its high impurity content (Pachauri and He, 2006; Thompson and He, 2006). The impurities composition of crude glycerol are varied with the type of catalyst used, the transesterification efficiency, recovery efficiency of the biodiesel, other impurities in the feedstock, type of alcohol used as well as the recovery efficiency of alcohol and catalyst (Yang et al., 2012). Crude glycerol is usually obtained from alkali-transesterification with a glycerol content about 50–60% (w/w) of glycerol as the mainly component and other impurities are 40–50% by weight (Gerpen, 2005; Manowattana et al., 2012; Saenge et al., 2011). The pH of crude glycerol is very high by pH 11–12 (Nicol et al., 2012) and it contains methanol in the range of 15–40% by weight (Hájek and Skopal, 2009; Manowattana et al., 2012; Thompson and He, 2006; Xu et al., 2012a). Normally, the glycerol layer is heated at 85°C for 1 h to remove the methanol before further utilization (Thompson and He, 2006). However, these impurities can be important considerations in the goal of bioconversion of crude glycerol to a higher value product (Nicol et al., 2012). Many aspects have been investigated to use crude glycerol as a sole carbon source for microbial growth because of their high value added metabolites production e.g. carotenoids (Manowattana et al., 2012; Saenge et al., 2011), single cell oil (André et al., 2010), lipids (Galafassi et al., 2012; Saenge et al., 2011) and organic acids (André et al., 2010).

Among many types of microorganisms, oleaginous red yeasts are the most efficient microorganism to use crude glycerol for increased growth rate and production of high value chemical metabolites (Meng et al., 2009). Nowadays, many researchers have investigated the ability of oleaginous red yeasts to produce those mentioned high value chemicals. Oleaginous red yeasts can accumulate both of high lipids content of more than 20% by dried cell weight and carotenoids with high β-carotene content in their cells (Meng et al., 2009; Zhang et al., 2011). Moreover, oleaginous red yeasts are one of the high potential natural carotenoids source because they are environmentally friendly compared to chemical production, able to meet the increasing demand for natural carotenoids and low production cost via a high efficiency fermentation process (Das et al., 2007). They can quickly accumulate lipids with the fatty acid composition similar to that of vegetable oils (Nigam and Singh, 2011) and have several advantages over vegetable oils e.g. short life cycle, low space demand and independence of location, seasons and climates (Bautista et al., 2012; Zhang et al., 2014).

In the recent year, the crude glycerol is interesting to be used as the carbon (C) source for dry cell weight (DCW), lipids and carotenoids productions by various types of oleaginous red yeasts. For example, carotenoids production by strain *Rhodosporidium paludigenum* (Yimyoo et al., 2011), *Sporobolomyces pararoseus* TISTR5213 (Manowattana et al., 2012), *Sporidiobolus pararoseus* (Valduga et al., 2014), *Sporobolomyces roseus* (Davoli et al., 2004) and *Xanthophyllomyces dendrorhous* (Certik et al., 2005) and lipids production by *Rhodotorula glutinis* TISTR5159 (Saenge et al., 2011), *Rhodosporidium toruloides* (Xu et al., 2012b) and *Rhodotorula graminis* (Galafassi et al., 2012). Effect of some chemicals found in crude glycerol e.g. methanol and free fatty acid and additive agents e.g. vegetable oil, organic acid and biosurfactants have been studied for their effects on lipogenesis and carotenogenesis of oleaginous red yeast (Mata-Gómez et al., 2014). For example, Xu et al. (2012b) reported that the lipids yield of *Rhodosporidium toruloides* decreased by 23.0% under the presence of methanol (0.8% w/w). Aksu and Eren (2005) reported that the supplementation of cotton seed oil 1.0% (w/v) could enhance the total carotenoids production yield of *Rhodotorula mucilaginosa* higher than in the absent of cotton seed oil by 45.82%. Similar to the results of Saenge et al. (2011) found that Tween 80 could also enhance the DCW, lipids and carotenoids productions of *Rhodotorula glutinis*.

Moreover, Kim et al. (2003) reported that adding 5.0 g/L of acetic acid could enhance astaxanthin production from 34.77 to 43.87 mg/L.

Therefore, demethanolized crude glycerol from biodiesel production plant was investigated as the potential C source for productions of lipids and carotenoids by an oleaginous red yeast *Sporidiobolus pararoseus* KM281507 (formerly *Sporobolomyces pararoseus* TISTR5213). The effect of some additive agent e.g. vegetable oils, biosurfactants and organic acids on lipids, fatty acid composition and carotenoids of strain KM281507 were also evaluated.

4.2 Material and methods

4.2.1 Identification of oleaginous red yeast strain

The identification of the oleaginous red yeast was performed by sequencing a genome fragment. The 26S rRNA gene was amplified by polymerase chain reaction (PCR) using primers F63 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and LR3 (5′-CCGTGTTTCAAGACGGG-3′). Amplification reactions were carried out in a 20 μL reaction volume under the following PCR cycling conditions: one cycle of denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and elongation at 72°C for 2 min, with a final extension step of 72°C for 5 min. DNA sequencing PCR reactions were carried out using the BigDye Terminator V3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA). The sequences chromatograms were assembled into one complete sequence using BioEdit Sequence Alignment Editor version 7.0.9.0 (Hall, 1999) and the sequence was compared to all known sequences in the GenBank database using the BLASTN 2.6.1+ program (Zhang et al., 2000). All sequences generated in this study were deposited in the GenBank database. This strain was identified as *Sporidiobolus pararoseus* under the accession number KM281507. This isolate was identified as *Sporidiobolus pararoseus* (Appendix E).

4.2.2 Microorganism and medium

The oleaginous red yeast *Sporidiobolus pararoseus* KM281507 was obtained from the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani 12120, Thailand. The inoculum preparation, production medium and cultivation condition was performed according to the method of Manowattana et al. (2012) The production medium containing (per liter); yeast extract 1.0 g, demethanolized crude glycerol 55.0 g, KH_2PO_4 5.5 g, $(NH_4)_2SO_4$ 5.3 g, K_2HPO_4 3.7 g, $MgSO_4.7H_2O$ 0.5 g, $MnSO_4.H_2O$ 0.2 g and NaCl 0.25 g (Appendix A).The initial pH of the medium was adjusted to 5.63 before sterilization at 121°C for 20 min (Manowattana et al., 2012) (Appendix A).

4.2.3 Raw materials

The biodiesel-derived crude glycerol was kindly donated by the Energy Research and Development Institute (ERDI), Chiang Mai University, Thailand. It was transferred into a separator funnel and allowed to settle overnight. The upper layer was discarded while the lower layer was used as the crude glycerol. The demetanolization of the crude glycerol was done by heating at 85°C for 1 h (Thompson and He, 2006). The concentration of glycerol in raw material and methanol were determined by using a high performance liquid chromatography (HPLC) (LC-10AT vp; Shimadzu, Japan) equipped with an Aminex HPX 87H column (300 × 7.8 mm; Bio-Rad, USA). The mobile phase of 5.0 mM H_2SO_4 was used as an eluent with a flow rate of 0.75 mL/min and the column thermostat was set at 40°C. Glycerol and methanol were detected by using a refractive index detector (RID, RID-10A; Shimadzu, Japan); in a linear gradient for 16 min, maintaining this proportion until the end of the run (André et al., 2010). Proximate analysis of ash, moisture and lipids contents of crude glycerol was determined according to the AOAC methods (Official Methods of Analysis of AOAC International, 2002).

4.2.4 Effect of additive agents

Effect of organic acids

Organic acids had been studied regarding their effect on lipids accumulation and carotenogenesis (Mata-Gómez et al., 2014). Therefore, in this study, the effect of some organic acids including formic acid (CH_2O_2), acetic acid ($C_2H_4O_2$), succinic acid ($C_4H_6O_4$) and citric acid ($C_6H_8O_7$) were studied. The concentration of the agents in the optimized medium was varied at 0.00, 0.10, 0.50 and 1.00% (w/v), respectively.

Effects of oils and derivatives

Various type of vegetable oils and derivatives e.g. free fatty acid (FFA) and biosurfactant derived from vegetable oil have been reported as enhancer of lipids and carotenoids productions by oleaginous red yeasts (Kim et al., 2006). In this study, Tween 20, Tween 40, Tween 60, Tween 80, oleic acid and olive oil at concentration of 0.00, 0.50, 1.00, 1.50 and 2.00% (w/v) were performed to achieve the maximum DCW, lipids and carotenoids contents of strain KM281507. The medium compositions and cultivation conditions were performed according to the optimal conditions obtained from the central composite design (CCD) which was described in the CHAPTER 3.

4.2.5 Analytical methods

Carotenoids extraction and determination

The carotenoids of *Sporidiobolus pararoseus* KM281507 were extracted by breaking the yeast cell. It was carried out in screw capped tube containing 10.0 mL acetone (Merck, Germany) and glass beads (3 mm; Superior, Germany). The extraction method and quantity analysis of the carotenoids were investigated by using HPLC equipped with a C18-column (5µm, 250m × 4.6 mm; Restek, France) following the suggestions provided by the method of Manowattana et al. (2012) The β-carotene and total carotenoids content were detected by using a UV-VIS detector (SPD-10A VP; Shimadzu, Japan), which operated at 454 nm. The β-carotene was identified by the β-carotene standard (Sigma, USA.)

Lipids extraction and determination

The lipids extraction from biomass was performed according to a modified method of Bligh and Dyer (1959). Briefly, lipids were extracted by breaking the yeast cell, carried out in screw capped tube with a mixture of chloroform:methanol (2:1, v/v) and glass beads for 30 min and sonicated at 70 Hz for 30 min (Elmasonic S60H; Elma Schmidbauer GmbH, Germany). The ruptured cells and extracted lipids were centrifuged at 6,000 rpm (4,146 *g*) at 4°C for 10 min (Hettich MIKRO 22R; Germany), and the clear supernatant was collected and the organic solvent was removed by evaporation under vacuum of 300 mm bar (Rotavapor R-3; Buchi, Japan). The lipids content was expressed as the volumetric of the extracted lipids in the culture broth (g/L).

Fatty acid composition of lipids

The compositions of fatty acid profile was investigated by derivatization of lipids to fatty acid methyl ester (FAME) following the method of Chaiyaso et al. (2012) The FAME was analyzed by gas chromatography with a flame ionization detector (GC-FID) (GC-2010; Shimadzu, Japan) equip with a HP-INNOWAX column (30 m × 0.25 mm, 0.25 µm film thickness; Agilent, USA.) with a split ratio of 100:1. Helium was used as the carrier gas with a 1.0 mL/min flow rate. The temperature program was 60°C (held for 2 min), 10°C/min to 200°C and 5°C/min to 240°C (held for 7 min). The GC-FID condition was performed following the EN14103:2011 method (McCurry, 2011).

The fatty acid profiles were further confirmed by GC-MS analysis which was analysed by the Science and Technology Service Center, Chiang Mai University (STSC-CMU), Chiang Mai 50100, Thailand. GC-MS (gas chromatography with mass spectroscopy; GC 7890A: MSD 5975C (EI): Agilent; USA), was performed with a scan parameter of 50–500 amu, MS quadrupole at 150°C and MS source at 230°C (modified method from McCurry (2011)) using a DB5-MS column (30 m × 0.25 mm, 0.25 µm film thickness) with a split ratio of 100:1. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The temperature program was as indicated above.

Biomass measurement

The biomass of oleaginous red yeast KM281507 was collected from the culture broth and centrifuged at 6,000 rpm (4,146 *g*) at 4°C for 10 min. The cell pellet was washed twice with *n*-hexane (LabScan, Thailand) and once with distilled water, then centrifuged and dried at 80°C for 24 h. After that, the dried cells were placed in desiccators until a constant weight was obtained (Manowattana et al., 2012).

Statistical analysis

All experiments were carried out as triplicate samples. The data were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test ($p<0.05$). The statistical software package SPSS v.17 was used in the analysis of the experimental data. Moreover, the experimental design, the statistical software package Design Expert 6.0.10 (Stat-Ease, Minneapolis, MN) was used in the design of the experiments, the analysis of the experimental data, and ingeneration of the response surface graphs. The significant model terms was conducted by *p*-value less than 0.05.

4.3 Results and discussion

4.3.1 Identification of oleaginous red yeast strain

Sporidiobolus pararoseus (accession number KM281507), formerly *Sporobolomyces pararoseus* TISTR5213 was reclassified based on nucleotide sequences of the 26S rRNA gene. The nucleotide sequences was submitted to BLAST and searched against the National Center for Biotechnology Information (NCBI) database (Figure 4.1).

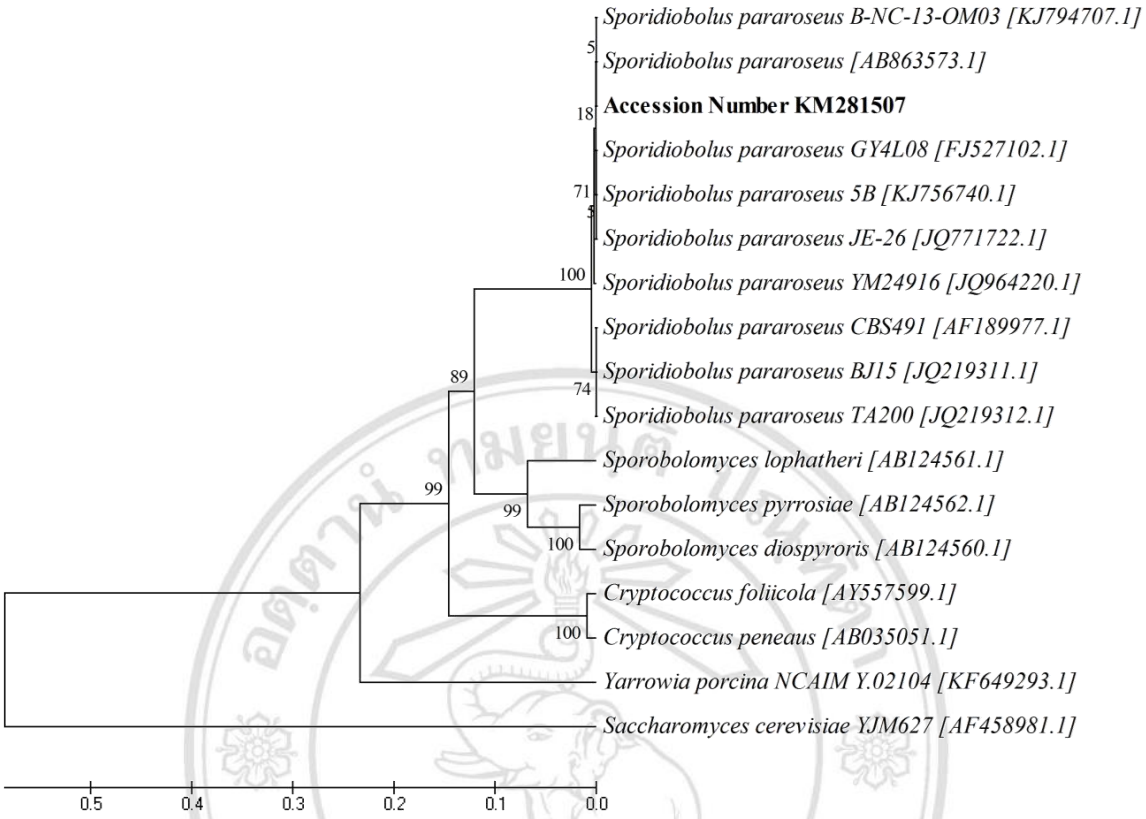

Figure 4.1 Phylogenetic tree constructed using the 26S rRNA gene sequence of *Sporidiobolus pararoseus*. The GenBank accession number of this strain is KM281507, and other yeast indicated in parentheses. The evolutionary history was inferred using the UPGMA method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches

4.3.2 Effect of additive agents

Effect of organic acids

Chemical and natural agents have been studied regarding their effects on lipids accumulation and carotenogenesis (Mata-Gómez et al., 2014). That organic acids are obtained from the intermediates of the Krebs cycle, which are usually carbon skeletons that can be directed to carotenoids and lipids biosynthesis (Sanchez et al., 2013). The organic acids consisting of formic, acetic, succinic and citric acids were used as additive agents in the optimized medium to enhance lipids and carotenoids productions of strain KM281507 (Figure 4.2). It was found that all of the organic acids enhanced the DCW and lipids productions in distinctive concentrations for each acid, in addition only formic acid did not improve carotenoids formation. The high DCW and lipids productions were increased to 12.24±0.08 g/L and 5.39±0.09 g/L, respectively, by addition of 0.5% (w/v) succinic acid. Moreover, this organic acid induced β-carotene and total carotenoids formation up to 50.09±0.74 and 64.92±0.78 mg/L, respectively, at 1.0% (w/v). While, low β-carotene (27.41±0.84 mg/L) and total carotenoids content (52.22±0.98 mg/L) under control condition were obtained. Lipogenesis and carotenogenesis are occurred in the cytoplasm (Sul and Smith, 2008). However, acetyl-CoA which is the precursor of biosynthesis of lipids and carotenoids derived from the breakdown of citric acid that under some conditions have been accumulated inside the mitochondria and then are secreted to the cytoplasm (Papanikolaou and Aggelis, 2011). Thus, addition of some Krebs cycle derived-organic acids e.g. citric and succinic acids might support the formation of acetyl Co-A as well as the biosynthesis of intracellular lipids and carotenoids (Papanikolaou and Aggelis, 2011).

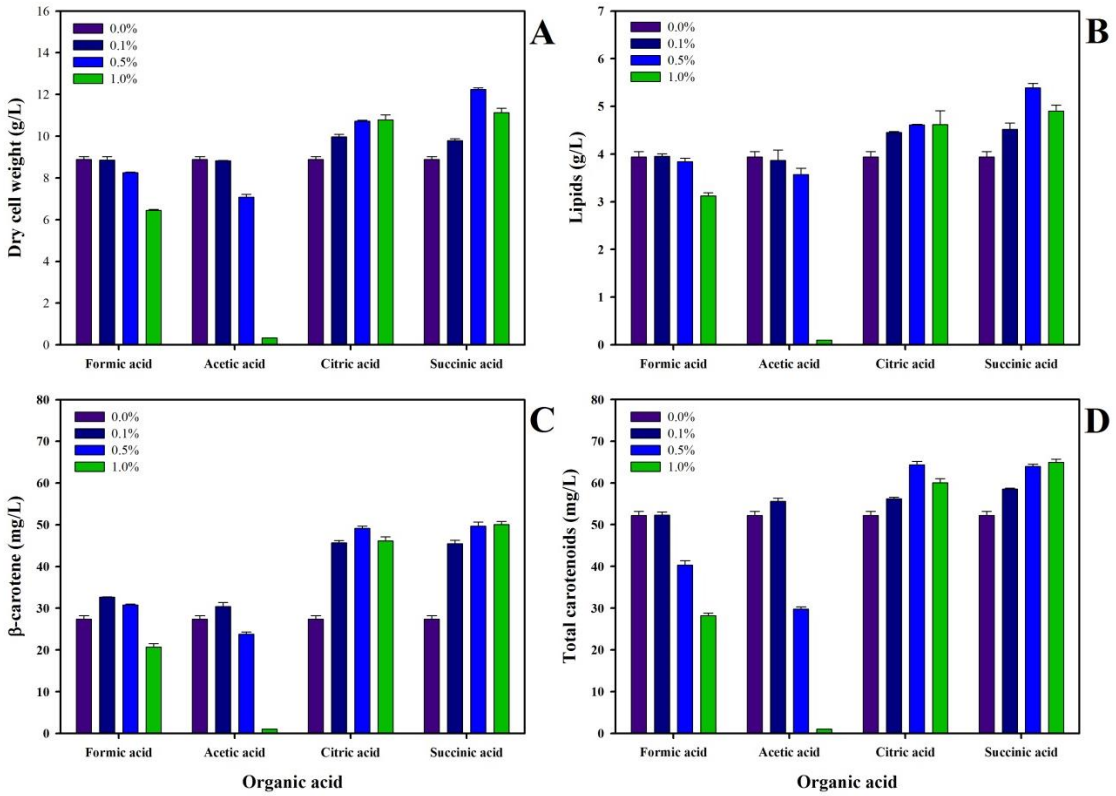

Figure 4.2 Effect of formic acid, acetic acid, citric acid and succinic acid on dry cell weight (A), lipids (B), β-carotene (C) and total carotenoids productions (D) of *Sporidiobolus pararoseus* KM281507 cultivated with demethanolized crude glycerol as a carbon source under optimal condition

Effect of fatty acid, oil and surface active agents

After the transesterification reaction of biodiesel production, unavoidably there are still some acylglycerols, and fatty acid alkyl esters (biodiesel) remaining in the aqueous glycerol phase. Moreover, oleic acid, a main fatty acid component in most of biodiesel feedstock has been found as the major free fatty acid in crude glycerol (Xu et al., 2012a). Some free fatty acids (FFA), oils and biosurfactants derived from vegetable oils have been reported as the enhancer the biosynthesis of lipids and carotenoids (Kim et al., 2006). Therefore, effect of biosurfactants, oleic acid and olive oil on the DCW, lipids, β-carotene and carotenoids productions of *Sporidiobolus pararoseus* KM281507 were investigated (Figure 4.3). Oleic acid was selected as the representatives of fatty

acid to investigate its effect on cell growth, lipids, β-carotene and total carotenoids accumulation of strain KM281507. It was found that 2.0% (w/v) of oleic acid concentration supported the cell growth and lipids productions to 11.47±0.15 g/L and 4.87±0.07 g/L, respectively. However, carotenoids production yield decreased with the addition of the oleic acid. Meanwhile, olive oil had high efficiency in supporting DCW, lipids, β-carotene and total carotenoids productions of strain KM281507. At concentration of 2.0% (w/v), was suitable to maximize the cell growth (14.96±0.19 g/L), while 1.5% (w/v) enhanced the lipids production to 6.40±0.09 g/L, and 0.5% (w/v) was good for carotenoids production (70.93±0.51 mg/L). Strain KM281507 could produce extracellular lipase (Manowattana et al., 2015), which might hydrolyze olive oil to free fatty acids and glycerol for further use as another carbon source (Pantazaki et al., 2010).

The surface active agents derived from vegetable oils including Tween 20, 40, 60 and 80 were not significant in increasing the cell growth and lipids productions (Figure 4.3). Tween 20 was used extensively for adding as an activator in microorganism medium (Saenge et al., 2011). The results found that Tween 60 could enhance carotenoids production higher than other biosurfactants with a maximum carotenoids concentration of 69.70±0.78 mg/L. Strain KM281507 has been reported as the lipase producer when cultivation in the presence of oils and derivatives e.g. Tween 20, Tween 40, Tween 60 and Tween 80 (Manowattana et al., 2015). Among biosurfactants used in this study, Tween 60 contains long chain fatty acid of stearic acid (C18:0) while palmitic acid (C16:0) and lauric acid (C12:0) are found in the Tween 40 and Tween 20, respectively. It might be after completely β-oxidation of Tween 60, resulting in higher amount of acetyl CoA. Moreover, oleic acid (C18:1) which found in Tween 80 might be oxidized to be acetyl CoA harder than stearic acid.

In comparison, total carotenoids production was not significantly different between addition of 0.5% (w/v) of either olive oil or Tween 60 (p>0.05). However, addition of olive oil could increase lipids production higher than Tween 60 by 53.11%. Among those studied additive agents, olive oil was good inducer for all parameters of DCW, lipids, β-carotene and total carotenoids productions by strain KM281507. At the appropriate concentration of olive oil, it could enhance the carotenoids biosynthesis by

35.83, β-carotene 98.57, DCW 68.47 and lipids 62.44%, respectively. Similar with the result of Aksu and Eren (2005) who also reported that the supplementation of cotton seed oil 1.0% (w/v) in the culture medium could increase the production of total carotenoids by *Rhodotorula mucilaginosa*.

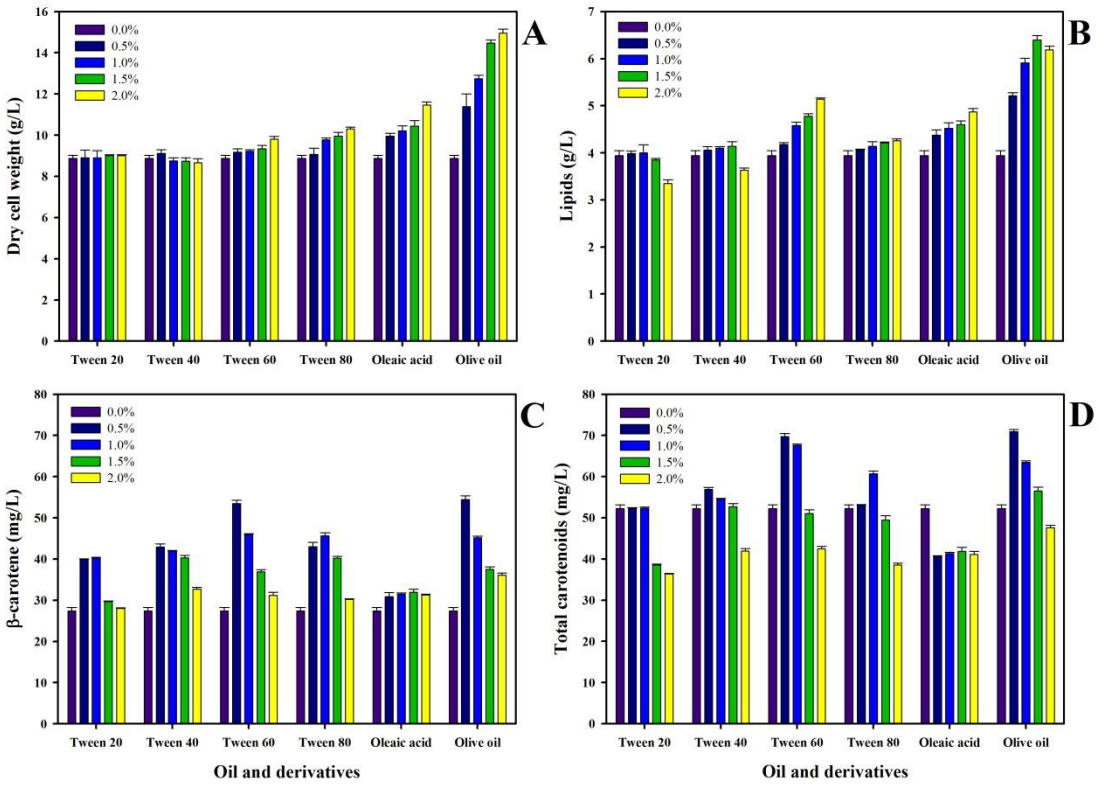

Figure 4.3 Effect of Tween 20, Tween 40, Tween 60, Tween 80, oleic acid (C18:1) and olive oil on dry cell weight (A), lipids (B), β-carotene (C) and total carotenoids (D) of *Sporidiobolus pararoseus* KM281507 cultivated with demethanolized crude glycerol as a carbon source under optimal condition

4.3.3 Fatty acid composition

Fatty acid composition of crude lipids obtained from strain KM281507 of each studied condition is shown in Table 4.1. The fatty acid profiles were composed of myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), while medium chain and long chain fatty acids were found as the minority. Normally, microbial oil differs from most vegetable oils and animal fats in being quite rich in unsaturated and polyunsaturated fatty acid (Meng et al., 2009). Similar to this study, the crude lipids obtained from *Sporidiobolus pararoseus* KM281507 cultivated under the optimized condition under the presence of 1.5% olive oil contained 78.20% of total unsaturated fatty acid with 75.08% of oleic acid while only 21.07% was saturated fatty acids. Similar to the results of Zhang et al. (2014) who found that crude lipids from *Rhodotorula glutinis* composed of oleic acid 51.30, linoleic acid 21.60, palmitic acid 16.50, stearic acid 3.70, palmitoleic acid 0.40 and myristic acid 0.70%, respectively, when glucose was used as a carbon source. While, oleic acid of 51.50% was found to be the predominant fatty acid in crude lipids from *Cryptococcus* sp. which was cultivated in corncob hydrolysate (Chang et al., 2015). Moreover, Saenge et al. (2011) found that cultivation of *Rhodotorula glutinis* in crude glycerol could increase the lipids with high content of oleic acid and linoleic acid. In contrast, glycerol decreased the unsaturated fatty acid content of crude lipids from *Rhodotorula graminis* (Galafassi et al., 2012). Similarly with the result of Xu et al. (2012b) who found that cultivation of *Rhodosporidium toruloides* in the medium containing crude glycerol, the oleic acid content in crude lipids was only 38.10% whereas palmitic acid content was increased to 29.10%.

The fatty acid profile and high oleic acid content of crude lipids from strain KM281507 was similar to that of vegetable oil indicating the high potential as the third biodiesel feedstock which can be applied in both of tropical and cold climate countries (Sarin, 2012). The degree of unsaturated fatty acid in biodiesel showed the excellent fuel properties at low temperature because this biodiesel had a lower melting point than the biodiesel derived from high saturated fatty acid content (Sarin, 2012).

Table 4.1 Effect of various types of additive agents on DCW and lipids productions, lipids content and fatty acid composition of *Sporidiobolus pararoseus* KM281507

Conditions	DCW (g/L)	Lipids (g/L)	Lipids content (% g/g DCW)	Fatty acid composition (%)						
				C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	Others
Optimum condition (CCD)	8.83±0.05^{g*}	3.94±0.11^{h}	44.37±1.25^{abcd}	0.51±0.02^{h}	13.00±1.10^{e}	1.03±0.01^{cd}	4.61±0.41^{de}	68.74±2.35^{b}	1.63±0.12^{a}	10.48±1.24^{c}
Effect of fatty acid and oil derivatives										
Olive oil (1.50%)	14.47±0.15^{a}	6.40±0.09^{a}	44.23±2.21^{abcd}	1.73±0.08^{cd}	14.19±1.21^{e}	1.79±0.01^{b}	5.15±0.48^{de}	75.08±3.54^{a}	1.33±0.11^{b}	0.74±0.48^{g}
Oleic (2.00%)	13.47±0.15^{b}	5.67±0.07^{b}	41.09±2.24^{d}	2.30±0.12^{b}	13.30±1.24^{e}	0.84±0.00^{de}	4.24±0.32^{de}	67.00±1.85^{bc}	0.43±0.05^{c}	11.89±1.02^{bc}
Effect of surface active agent										
Tween 20 (1.00%)	8.90±0.35^{g}	4.00±0.17^{h}	44.94±1.45^{abc}	5.25±0.47^{a}	20.30±1.80^{c}	0.46±0.00^{ef}	7.74±0.56^{c}	61.11±1.74^{e}	0.00±0.00^{d}	5.15±0.42^{e}
Tween 40 (1.50%)	8.73±0.17^{g}	4.14±0.10^{gh}	47.42±0.69^{a}	0.86±0.03^{g}	18.20±1.54^{cd}	2.73±0.14^{a}	3.90±0.31^{ef}	65.41±2.14^{bcd}	0.00±0.00^{d}	8.90±0.49^{d}
Tween 60 (0.50%)	10.18±0.17^{e}	4.18±0.04^{gh}	41.06±2.94^{d}	1.59±0.09^{cde}	16.05±1.21^{de}	0.82±0.02^{de}	5.55±0.49^{d}	64.61±2.18^{cde}	0.00±0.00^{d}	11.37±0.98^{c}
Tween 80 (2.00%)	10.30±0.10^{e}	4.26±0.04^{fg}	41.36±2.06$_{cd}$	1.12±0.08^{fg}	20.00±1.84^{c}	0.63±0.01^{de}	2.78±0.27^{f}	62.36±2.16^{de}	0.00±0.00^{d}	13.10±1.08^{b}
Effect of organic acid										
Formic acid (0.10%)	11.06±0.17^{d}	5.05±0.06^{d}	45.66±1.38^{ab}	1.38±0.07^{ef}	34.54±2.35^{a}	0.85±0.02^{de}	13.78±1.11^{a}	40.70±1.98^{g}	0.00±0.00^{d}	8.76±0.64^{d}
Acetic acid (0.10%)	9.83±0.01^{f}	4.47±0.21^{ef}	45.47±2.53^{ab}	1.47±0.10^{de}	27.72±2.18^{b}	1.46±0.90^{bc}	12.35±1.01^{b}	43.82±1.84^{fg}	0.00±0.00^{d}	13.18±0.95^{b}
Citric acid (1.00%)	10.79±0.24^{d}	4.62±0.29^{e}	42.82±1.39^{bcd}	1.77±0.11^{c}	33.82±2.47^{a}	1.02±0.10^{cd}	14.64±1.20^{a}	46.46±1.47^{f}	0.00±0.00^{d}	2.29±0.09^{f}
Succinic acid (0.50%)	12.24±0.08^{c}	5.39±0.09^{c}	44.04±2.46^{abcd}	1.04±0.08^{g}	24.85±2.14^{b}	0.00±0.00^{f}	11.63±1.00^{b}	45.84±1.48^{f}	0.00±0.00^{d}	16.63±1.11^{a}

*Means and standard deviations of triplicate samples

Value with different significance according to the statistical analysis Duncan's multiple range test (p<0.05)

4.4 Conclusions

Sporidiobolus pararoseus KM281507 had a high potential for bioconversion of demethanolized crude glycerol to lipids and carotenoids productions. Moreover, olive oil (0.5–1.5%) had high efficiency as an activator for dramatically enhancing of biomass, lipogenesis and carotenogenesis of strain KM281507. Oleic acid (75.08% of total fatty acid) was a main fatty acid of lipids from strain KM281507. Therefore, the results of this study indicated that oleaginous red yeast strain KM281507 had a great potential microbial source for the third biodiesel renewable feedstock and natural source of carotenoids.