Renewable Biodiesel Production from Oleaginous Yeast Biomass Using Industrial Wastes

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Abstract. The accumulation lipid from oleaginous microorganisms is recognized as a second generation fuel. Biooil is known to as intracellular product of oily yeast utilizing various carbon substrates and converting different quantities of lipids in the form of triacylglycerols. This second generation fuel can be used to make biodiesel via a transesterification process. This study investigated the morphological characteristics of eight strains of Thai oleaginous yeasts via microscopy and analyzed the fatty acid profiling of yeasts cultured in three carbon sources: glucose, sugar cane molasses and crude glycerol in order to estimate biodiesel properties. To approach this goal, batch fermentations were used to culture eight yeast strains, *Rhodosporidium toruloides* TISTR 5123, TISTR 5154, TISTR 5149, *Yarrowia lipolytica* TISTR 5054, TISTR 5151, TISTR 5621, *Rhodotorula glutinis* TISTR 5159 and *Rhodotorula graminis* TISTR 5124 for 96 h under 30°C at 250 rpm. Result revealed that eight yeast strains contained significant amounts of fatty acids and lipids and accumulated mainly palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C 18:1) and linoleic acid (C18:2), and they are suitable for the production of biodiesel. Fatty acid productions and profiles indicated that these yeast strains can be potentially used as the triacylglycerols producers for biodiesel production.

1 Introduction

Currently, soybean, rapeseed and palm oil are the main feedstock for biodiesel production in America, Europe and Asia [1]. These feedstocks are commonly applied to biodiesel fuel production via transesterification. Alternatively, oleaginous microorganisms are including bacteria, yeast, fungi and algae. Microorganisms are commercially used as interesting sources for biooil production [2]. Especially for oleaginous yeast which is the well-known microbial oil organism because they produced more than 20% of their biomass into intracellular lipid.

These microorganisms produce triacylglycerol and many type of fatty acids depending on cultivation media and stress under the growth. The similarity of triacylglycerols (TAGs) of the biooil from vegetable oils have increasing the interest to use these fatty acid compositions to further synthesis a mixture of fatty acid methyl esters (FAMEs) in to biodiesel [3]. Soccool *et al.*, 2017 [4] reported that approximately a hundred microorganisms have already been recognized as producers. Some examples include *Rhodotorula gracilis*, *Crytococcus curvatus*, *Yarrowia lipolytica*, *Rhodosporidium toruloides*, *Trichosporon capitatum*, *Cunninghamella echinulate, Candida curvata* and *Mucor* sp. [4-6]. Among

these strains, *Rhodosporidium toruloides* produced lipids at 55-68% [7, 8]. For *Yarrowia lipolytica* strains*,* their intracellular lipids are approximately 30-43% [9, 10]. Moreover, Meng, 2009 reported that *Rhodotorula gracilis* contained 72% of lipid in their biomass. Most of these microorganisms produced biolipids more than 30% that have been using many sugars as raw materials for biodiesel production [3, 11, 12]. To increase the feasibility to produce biooil, finding other glucose sources for cultivation from industrial wastes, such as molasses and crude glycerol should be explored.

Several investigations have been explored opportunity to use molasses and crude glycerols as the main substrates for production [13, 14]. The important parameters of feedstock to produce biodiesel is not only the total quality of fatty acid accumulation during the cultivation, but also the quantity of the fatty acid. In terms of yeast species, nutritional compositions (as carbon/nitrogen), cultivation time and temperature play a pivotal role in lipid content and each type of fatty acid composition to the biodiesel quality [1, 15, 16]. Type of microorganism, substrate and culture condition significantly affect composition of fatty acid, a basic factor that influence biodiesel properties [17].

To maintain quality of biodiesel, several properties must be evaluated and compared with the international

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biodiesel standard specification. Some of these properties include viscosity, specific gravity, cloud point, cetane number, iodine number and higher heating value (HHV). Quality standard of biodiesel have been defined by several countries such as American Standards for Testing Materials (ASTM 6751-3), European Union (EN 14214) and Germany (DIN 51606).

Depending upon the global standard of biodiesel quality, chain length, degree of unsaturation and branching are the key characteristics. They contribute to determine the quality of biodiesel. The comparison of biodiesel of our study yeast strains under two different types of industrial wastes have to be evaluated to compare with European (MS14214) and American standards (ASTM D6751). In this study, eight Thai screening oleaginous yeasts were studied for the ability to grow and produce intracellular lipids using industrial wastes (molasses and crude glycerol) as a renewable energy, in order to develop a microbial process for TAGs production. Fatty acid profiling from the utilization of various yeast strains using molasses, crude glycerol and glucose were reported.

2 Materials and methods

2.1 Industrial waste materials

Molasses was obtained from Singburi Sugar Co., Ltd. (Singburi, Thailand). Preliminary analysis of composition composed of 32 % sucrose, 14 % glucose 16% fructose, 10% total nitrogen, 10% of trace minerals as 0.5 % SiO_2, 3.5% K_2O, 1.5% CaO, 0.1% MgO, 0.2% P_2O_5, 0.2% Fe_2O_3, 1.6% SO_3 0.4% Chlorides with 20% water. Total soluble solid contained 88 ^{0}Brix, pH 5.4. Crude glycerol was received from Pathum Vegetable Oil Co., Ltd, Thailand. Composition composed of 85.90 % glycerol, 4.8% NaCl, 9.9 % moisture content, 0.04 % fatty acid and ester, pH 6.4 with specific gravity of 1.2564 g.cm^{-3}.

2.2 Microorganism

Rhodosporidium toruloides TISTR 5123, 5154 and 5149 *Yarrowia lipolytica* TISTR 5054, 5151, 5621, *Rhodoturola glutinis* TISTR 5159 and *Rhodoturola graminis* TISTR 5124 were purchased from Thailand Institute of Scientific and Technological Research. Yeast culture incubated in yeast malt agar at 30°C for 48 h was observed and photographed by using the light microscopy with amplification at 1000x.

2.3 Inoculum preparation

Yeast strains were revived from lyophilized vials and grown on yeast malt agar (YMA) plates. Seed preparation was transferred from the stock solution to YMA slants and incubated for 2 days at 30°C. Following this period, colonies were transferred into the yeast malt broth and incubated for 48 h before transferring the 10% of inoculums in 100 mL of media: 10 g/L glucose, 3 g/L

yeast extract, 3 g/L malt extract and 5 g/L peptone. Cultures were then incubated at 30°C in a shaking incubator with the shaking speed of 200 rpm for 48 h. For carbon source comparison, glucose, molasses and crude glycerol, the compositional of modified media were prepared as 20 g/L carbon source, 1 g/L yeast extract, 1g/L $(NH_4)_2SO_4$, 0.5g/L KH_2PO_4. A series of 100 mL modified media experiments were inoculated with 1% v/v inoculum then incubated in shaking incubator at 200 rpm at 30°C for 48 h. Each sample was collected after centrifugation at 6,000 rpm for 15 min and analyzed in triplicate. Biomass was determined by measuring the dry weight.

2.4 Analytical methodology

2.4.1 Cell growth

5 mL cultures were harvested from each media by centrifugation at 6,000 rpm for 15 minutes then discarded the supernatant. All precipitate was kept and wash with 0.85% saline water subsequently with the same volume of water. All samples were dried at 80°C. The biomass was determined gravimetrically.

2.5.2 Determination of lipids and the fatty acid profile (FAME)

Lipid from dry cell weight biomass were extracted according to the method of Bligh and Dyer (1959) [18]. The extracted lipid fraction was esterified to obtain the fatty acid methyl esters. Analyses were performed in triplicate. FAME were analyzed in a gas chromatography equipped with FID (flame ionization detection) and the capillary column DB-Wax (30m length x 0.25 mm internal diameter x 0.2 μm film thickness). Temperatures of the detector and injector were 230°C at 3°C/min. The following temperature program was used for the separation of FAME: 100°C for 1 minute, with a gradual increase of 2°C per minute until the final temperature of 230°C, where it was held for 18 minutes. The carrier gas was helium with constant flow. Volume of injection was 1 microliter with split rate of 1:100. The identification of FAME was evaluated using Supelco 37 Component Fame Mix, C4-C24 alkane as standard.

2.6 Biodiesel criteria

The biodiesel properties based on the quality and the percent composition of FAME from extracted lipid material from intracellular oleaginous yeast in different culture condition. The evaluation was performed on the calculation of individual characteristic properties of biodiesel [1].

The quality factors evaluated were viscosity, specific gravity, cloud point, cetane number, iodine number and higher heating value. The parameters were compared with the limit values stipulated according to standard ASTM-D6751 (EUA) and EM 14213 (Europe).

Average unsaturation (AU) was calculated from the compositional of fatty acid analysis as illustrate in Fig. 2-4. Each property was calculated using Equation. (1)-(7)

$$AU = \sum N \times C_i \qquad (1)$$

$$\text{Viscosity} = -0.6316\ AU + 5.2065 \qquad (2)$$

$$\text{Specific gravity} = 0.0055 AU + 0.8726 \qquad (3)$$

$$\text{Cloud point} = -13.356 AU + 19.994 \qquad (4)$$

$$\text{Cetane number} = -6.6684 AU + 62.876 \qquad (5)$$

$$\text{Iodine number} = 74.373 AU + 12.71 \qquad (6)$$

$$HVV = 1.7601\ AU + 38.534 \qquad (7)$$

Where N is the number of carbon-carbon double bonds of unsaturated fatty acids while C_i is the concentration (mass fraction) of the component.

3 Results and discussion

3.1 Oleaginous yeast morphology

R. toruloides, R. glutinis and R. graminis strains were notable oleaginous yeast because they were non-pathogenic species and good producer of carotenoides. They belong to red color basidiomycete. These strains showed different shade of pigments in their colony. Results showed that *R. toruloides* TISTR 5123 and TISTR 5154 revealed orangish-red color with ovidal to spherical cell shape Figure 1 (B1, B3, B7 and B8) For. *R. toruloides* TISTR 5149, it showed pink, circular, smooth, moist colonies. For *Y. Lipolytica*, which belong to Ascomycota division, showed yellowish to creamy white, irregular, wrinkled, moist colonies especially *Y. lipolytica* TISTR 5151 (Fig. 1).
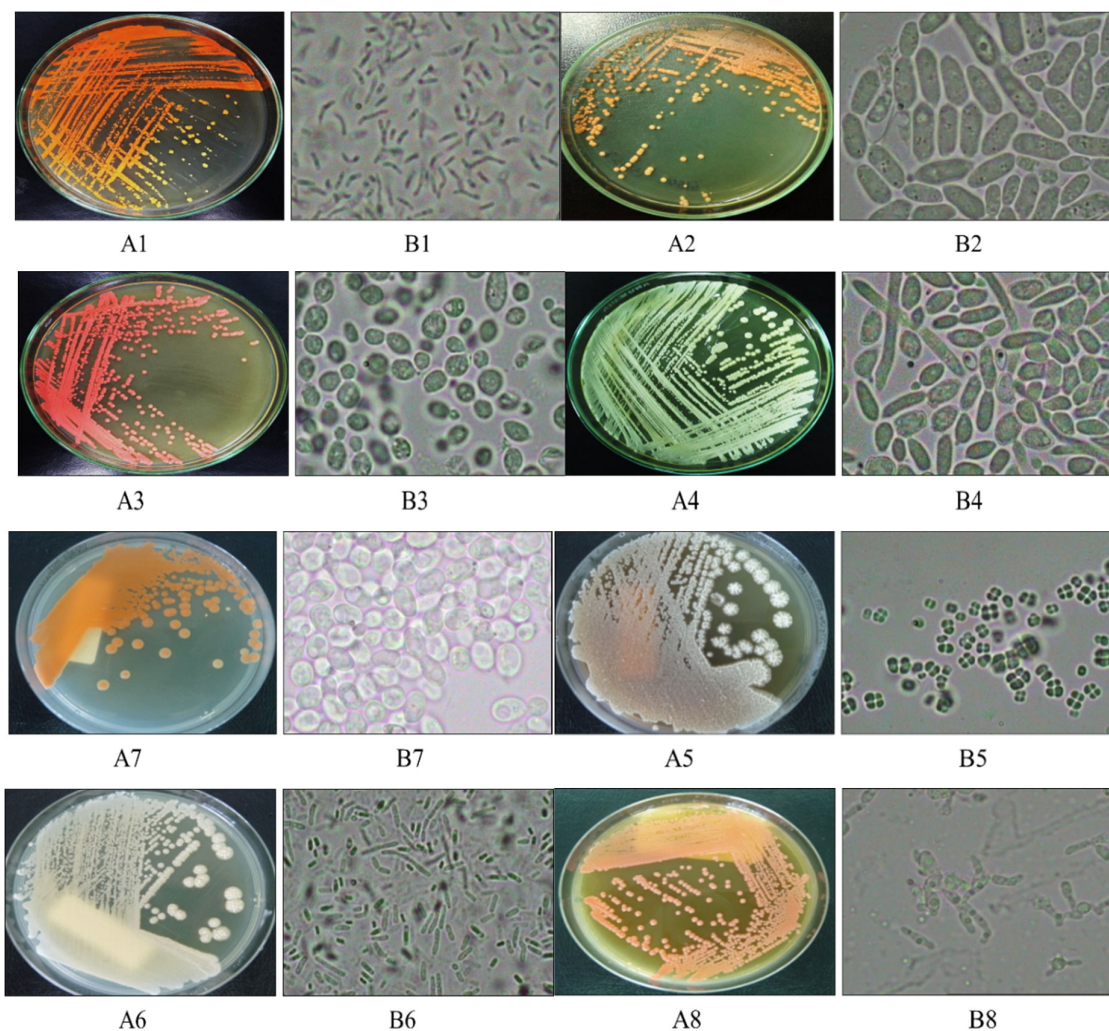

Fig. 1. Oleaginous yeasts (A) in YMA media and (B) on microscopy with magnification of 1000X : (1) *R. toruloides* TISTR 5123, (2) *R. toruloides* TISTR 5154, (3) *R. toruloides* TISTR 5149, (4) *Y. lipolytica* TISTR 5054, (5) *Y. lipolytica* TISTR 5151, (6) *Y. lipolytica* TISTR 5621, (7) *R. glutinis* TISTR 5159 and (8) *R. graminis* TISTR 5124 (Towijit *et al*, 2014, [19].

To choose the suitable oleaginous yeast from eight oleaginous yeasts, the biomass production yield and cellular lipid content were determined as shown in Table 1. For our chosen strains of oleaginous yeasts, they are scattered among different groups in phylum Ascomycota and Basidiomycota. However, results showed all strains of *Y. lypotica* produced more biomass more than *R. toruloides, R glutinis* and *R. gramini,* when grown in glucose. The yeast strain *Y. lipotica* TISTR 5054 gave the most biomass (3.469 ± 0.301 g/L); however, *R. glutinis* TISTR 5159 produced the least biomass (1.559 ± 0.279 g/L) in glucose. Maximum intracellular lipid yield was attained by *Y. lipotica* 5151 (3.377 ± 0.259g/L) followed by *Y. lipotica* 5054 (0.595 ± 0.034 g/L), *Y. lipotica* 5621 (0.565 ± 0.115 g/L) and *R. toruloides* TISTR 5149 (0.450 ± 0.044 g/L). *R. toruloide* TISTR 5154 gave the lowest lipid yield (0.168 ± 0.014 g/L) as shown in Table 1.

Once considered the industrial carbon source as molasses, the maximum cellular lipid content was attained by *R. toruloide* TISTR 5154 (32.35%) followed by *Y. lipotica* 5054. (21.51 %), *Y. lipotica* 5151 (21.0 9%). *R. toruloide* TISTR *5149* had the lowest lipid content 10.24%. Comparison of crude glycerol growth in Table 1, only *R.graminis* could be considered as oleaginous yeast in this experiment. The lowest lipid productivity was found in *Y. lipotica* TISTR 5054. The high lipid percentage were varied in *R. graminis* TISTR 5124, *R. toruloides* TISTR 5123 and *R. toruloides* TISTR 5149. Our strains yield both biomass and lipid lower than other researchers reported. *R. toruloides* is a red basidiomycete, isolated from conifer, and is capable of accumulating up to 76% of lipids in glucose [20]. *R. glutinis* is able to accumulate up to 66% of it dry weight as lipids and can reach 72% liquid accumulation with glucose as the carbon source [21].

For cultivation in glucose, molasses and crude glycerol, the fatty acid profiles of each yeast were shown in Fig. 2-4. All strains contained mainly palmitic acid (C16:0), steric acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) acids. Fatty acids of all strains contained lauric acid (C12:0). Six strains of oily yeast contained intracellular lipid more than 30% in glucose cultivation. The highest lipid production was *Y. lipolytica* TISTR 5621 yield more than 50% palmitic acid of those fatty acids in glucose cultivation.

The suitable selection for using molasses as the substrate was *R. toluloides* TISTR 5124 because of its yield of more than 40% C16:0, while the production of intracellular lipid cultured in glycerol showed that *Y. lipolytica* TISTR 5151 yielded over 40% of palmitic acid. Results revealed that C18:0, C18:1 and C18:2 varied among strains and carbon sources. The suitability of microorganism-based biodiesel for application as a fuel requires certain properties similar to traditional biodiesel, and need to be fulfilled with the specification by internationals standards. These parameters may influence by the individual characteristics of fatty acid methyl ester (FAME). When fatty acids have little saturation, for example C16:0, C18:0, C18:1, C18:2 and C18:3, they are suitable for the production of biodiesel; on other hand, when rich in polyunsaturated fatty acids with four or more unsaturations, they become unsuitable due to their accountability to oxidation [22, 23].

Table 1. The biomass and lipid concentration of eight oleaginous yeasts in different carbon sources.

| Strains | Carbon source | | | | | |
| | Glucose | | Molasses | | Crude glycerol | |
	Biomass (g/L)	Lipid (g/L)	Biomass (g/L)	Lipid (g/L)	Biomass (g/L)	Lipid (g/L)
R. glutinis TISTR 5154	2.139±0.297	0.168±0.014	0.544±0.019	0.176±0.021	0.376±0.002	0.060±0.004
R. glutinis TISTR 5159	1.559±0.279	0.449±0.005	1.848±0.038	0.264±0.050	0.311±0.002	0.051±0.009
R. toruloides TISTR 5123	2.013±0.116	0.278±0.027	1.046±0.087	0.186±0.033	0.4526±0.005	0.076±0.004
R. toruloides TISTR 5149	2.305±0.137	0.450±0.044	2.363±0.009	0.242±0.038	0.3667±0.003	0.061±0.003
R. graminis TISTR 5124	1.429±0.154	0.399±0.045	1.529±0.046	0.302±0.067	0.398±0.008	0.086±0.004
Y. lipolytica TISTR 5054	3.469±0.301	0.595±0.034	1.417±0.174	0.319±0.054	0.602±0.049	0.074±0.005
Y. lipolytica TISTR 5151	3.377±0.259	0.829±0.049	2.214±0.082	0.467±0.046	1.189±0.534	0.167±0.003
Y. lipolytica TISTR 5621	3.306±0.167	0.565±0.115	2.274±0.092	0.428±0.064	1.105±0.157	0.139±0.004
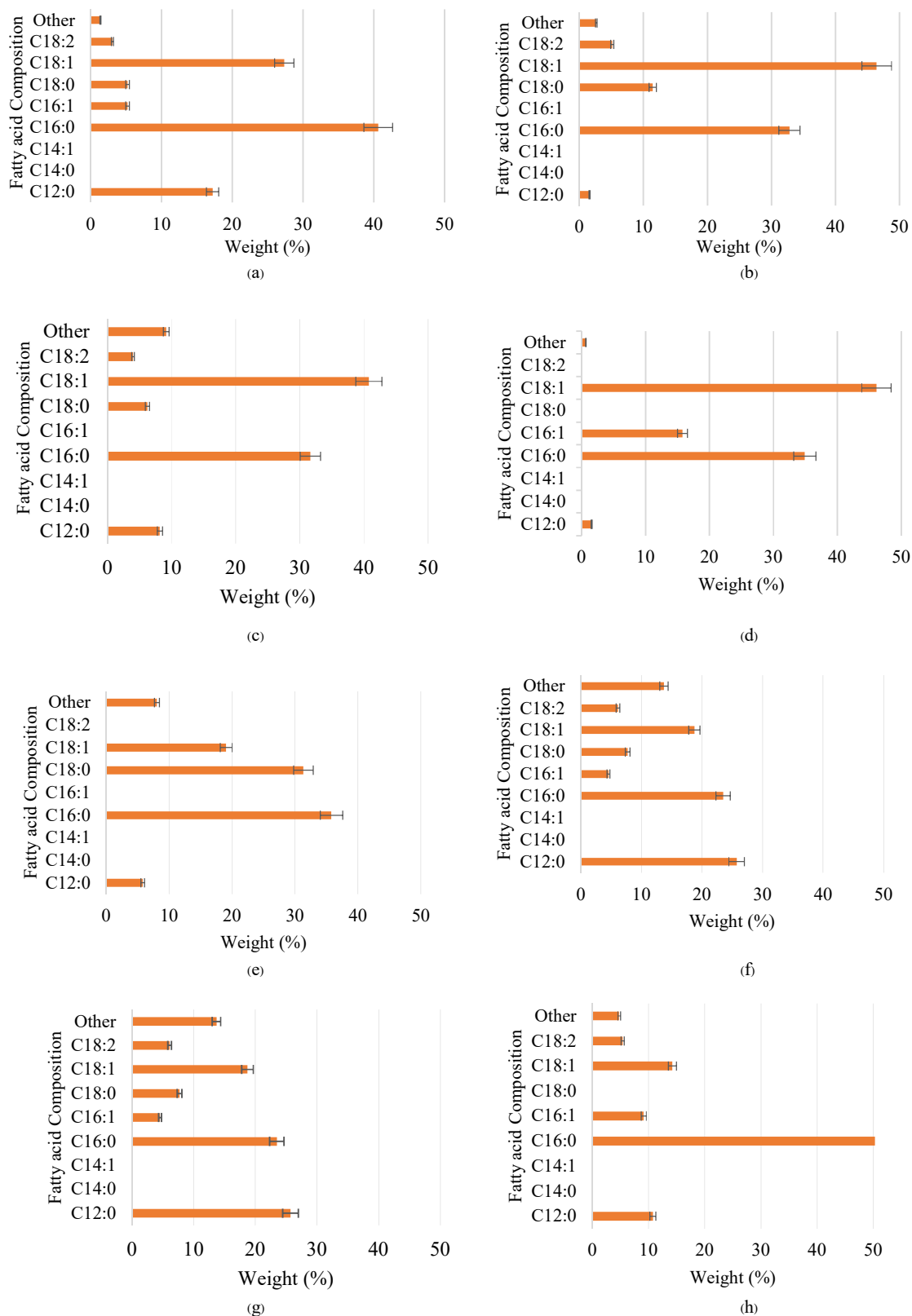
Fig.2. Compositional profiles of fatty acids from yeast cultivation in glucose (a) *R. toluloides* TISTR 5154, (b) *R. toluloides* TISTR 5159, (c) *R. toluloides* TISTR5123, (d) *R. toluloides* TISTR5149 (e) *R. toluloides* TISTR 5124, (f) *Y. lipolytica* TISTR 5054, (g) *Y. lipolytica* TISTR5151, and (h) *Y. lipolytica* TISTR 5621.

Fig. **3**. Compositional profiles of fatty acids from yeast cultivation in molasses (a) *R. toluloides* TISTR 5154, (b) *R. toluloides* TISTR 5159, (c) *R. toluloides* TISTR5123, (d) *R. toluloides* TISTR5149 (e) *R. toluloides* TISTR 5124, (f) *Y. lipolytica* TISTR 5054, (g) *Y. lipolytica* TISTR5151, and (h) *Y. lipolytica* TISTR 5621.
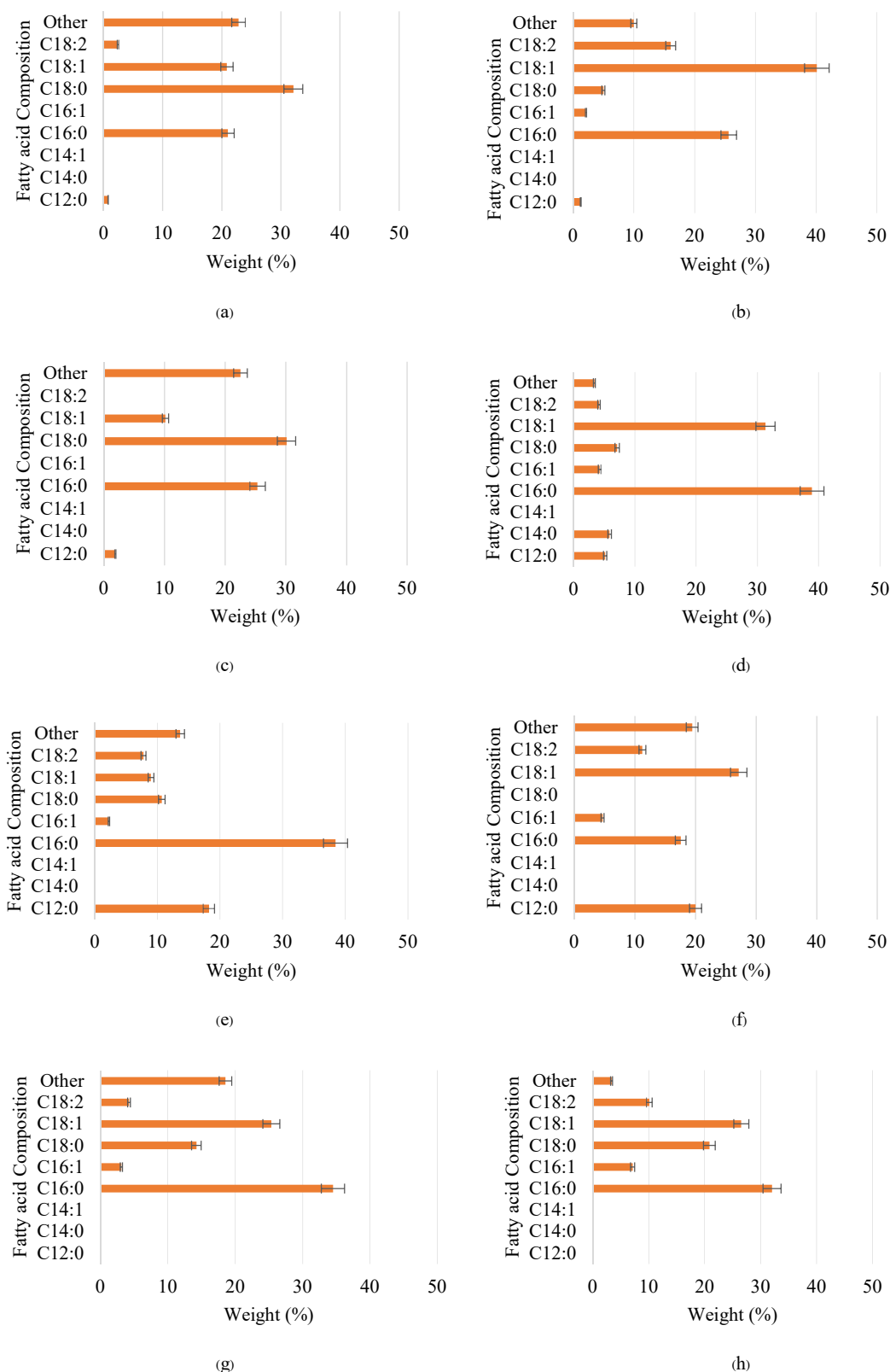
Fig. **4**. Compositional profiles of fatty acids from yeast cultivation in glycerol (a) *R. toluloides* TISTR 5154, (b) *R. toluloides* TISTR 5159, (c) *R. toluloides* TISTR5123, (d) *R. toluloides* TISTR5149 (e) *R. toluloides* TISTR 5124, (f) *Y. lipolytica* TISTR 5054, (g) *Y. lipolytica* TISTR5151, and (h) *Y. lipolytica* TISTR 5621.
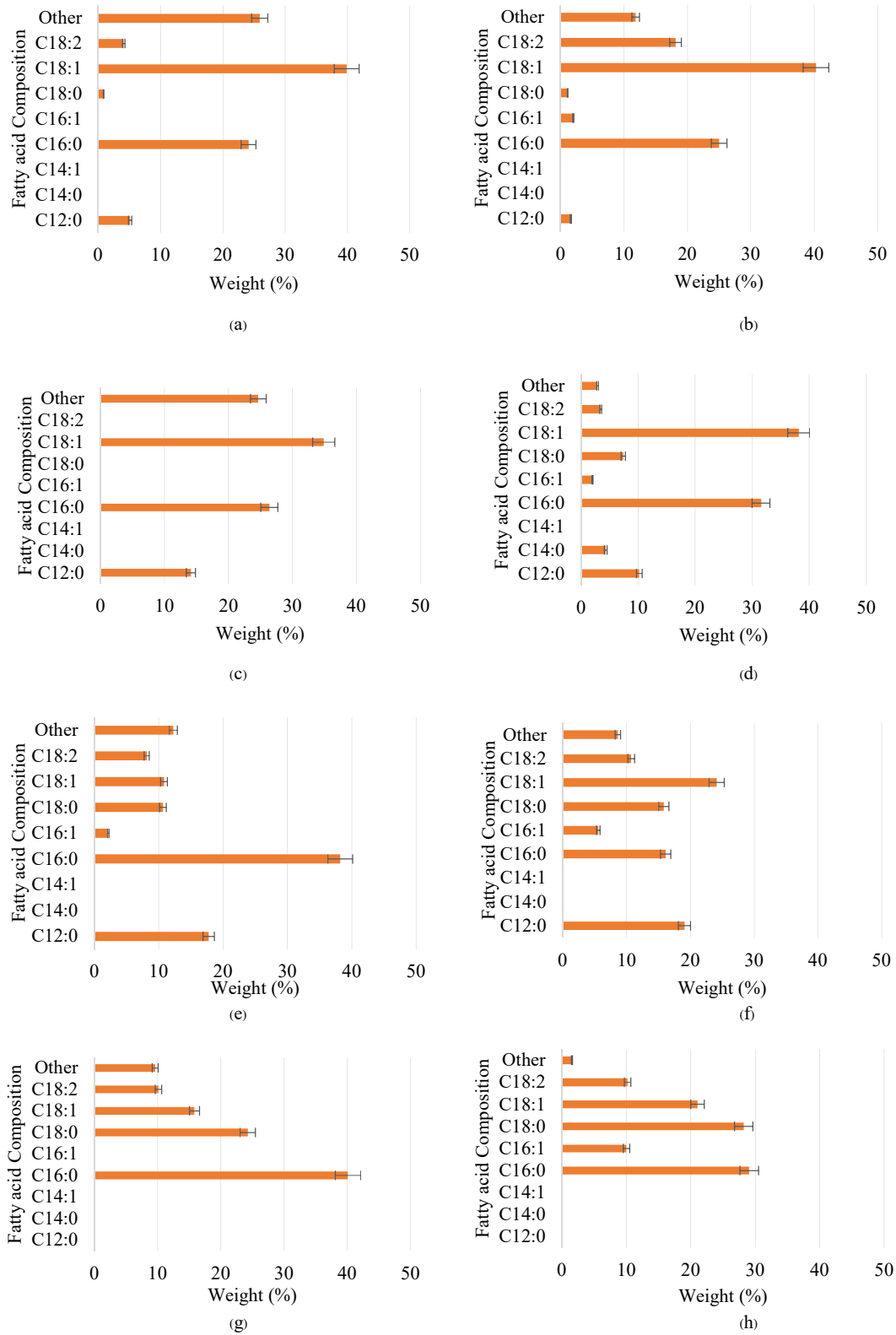
In our study biodiesel properties were determined using the concentration (mass fraction) of the component, and the number of carbon-carbon double bonds of unsaturated fatty acids (Table 2-4). Results showed they were suitable for biodiesel production because they were rich in composition of C16:0, C18:0, C18:1, and C18:2. Biofuels used in Europe are characterized by certain specification, which the content of linolenic ester acid (C18:2n6c) should not exceed 12% [24]. The main properties such as viscosity, specific gravity, cloud point, cetane number, iodine number and higher heating value (HHV) were directly reported to FAME. For biodiesel properties, a measurement of resistance to flow of a liquid results were showed in kinematic viscosity value [24], our data showed that viscosities of eight oleaginous yeast were within a narrow range of 4.00-5.30 mm²/sec of all carbon sources. Our yeast strains have slightly lower viscosity compared to palm oil, at 4.61 mm²/sec. The ASTM D6751 viscosity specification of 1.9-6.0 mm²/sec was satisfied by all eight oleaginous yeasts. Additionally, double bond configuration influence viscosity with lower effects [25]. The more restrictive EN 14214 specification of 3.5-5.0 mm²/sec excluded almost yeast strains, except *Y. lipolytica* TISTR 5621 and *R. graminis* TISTR 5124 (Table 3).

Viscosity value normally related to the degree of unsaturation with higher unsaturation resulting in lower viscosity. For specific gravity varied within a narrow range of 0.8726-0.8750 for all eight yeast strains. Palm-derived FAME had the lowest specific gravity value of 0.873, while jatropa oil yielded 0.876 (Table 2-4). Biodiesel of our selected yeasts would meet the specification of the EN 14214 standard. For ASTM D6751 standard did not include a specific density. Cloud point showed similar pattern across the strains and carbon sources. Low temperature performance as represented in cloud point value showed satisfaction of biodiesel users. Normally, biodiesel from palm oil showed the poorest performance.

This cloud point can be used to determine the suitability of biodiesel fuels in use. However, both U.S. and European biodiesel standard did not include explicit specification for this property.

Cetane number is the influential value that showed a measurement of an autoignition quality characteristics. These cetane values for all eight yeast cultures in all carbon sources easily overcome the ASTM miminum specification of 47 with the highest cetane value being observed for oleaginous yeast grown on glucose (Table 3). The cetane numbers of FAME fuels obviously varied with average degree of unsaturation of fatty acid. Another properties, iodine value (IV) was a measurement for determination of FAME unsaturation. Our experiment showed that ASTM D6751 did not include a specification for IV. All strains showed in Table 3-5 passed the EN 14214 that has a maximum IV of 120 mgI₂/100g FAME. Lastly, higher heating value related to the amount of the fatty acid carbon chain increased the mass fraction of oxygen decreased; therefore, the heating value increased [26-28].

4 Conclusion

The fatty acid profiles of eight oleaginous yeast strains were summarized and compared with commercial compositional variability. They contained C16:0, C18:0, and C18:1 and C18:2 and higher levels of polyunsaturated fatty acid that potentially considered to be an alternative feedstock under cultivation with three different carbon sources, glucose, molasses and crude glycerol. However, more researches on optimization of cultivation condition including varying carbon to nitrogen ratio, cultivation time and temperature have to be further investigated.

Table 2. Comparison of biodiesel properties from cultured oleaginous yeasts in glucose.

Yeast strains	Viscosity (mm²/s)	Specific gravity	Cloud point (°C)	Cetane number	Iodine number	HHV (MJ/kg)
Rhodosporidium toruloides TISTR 5123	5.21	0.8726	19.99	62.88	12.71	38.53
Rhodosporidium toruloides TISTR 5154	5.21	0.8726	19.99	62.88	12.71	38.53
Rhodosporidium toruloides TISTR 5149	5.21	0.8726	19.99	62.88	12.71	38.53
Yarrowia lipolytica TISTR 5054	5.21	0.8726	19.99	62.88	12.71	38.53
Yarrowia lipolytica TISTR 5151	5.04	0.8740	16.57	61.17	31.77	38.99
Yarrowia lipolytica TISTR 5621	5.14	0.8732	18.63	62.19	20.33	38.71
Rhodotorula glutinis TISTR 5159	5.21	0.8726	19.99	62.88	12.71	38.53
Rhodotorula graminis TISTR 5124	5.21	0.8726	19.99	62.88	12.71	38.53
Palm oil[a]	4.61	0.8730	14.0	61.9	54.0	40.6
Jatropa oil[a]	4.48	0.8789	4.67	55.23	98.20	40.55
	(4.75)	(0.876)	(5)	(55.7)	(109.5)	(40.7)
US biodiesel standard ASTM D6751[a]	1.9-6.0	-	-	47 min	-	-
EU Biodiesel standard EN 14214[a]	3.5-5.0	0.86-0.9	-	51 min	120 max	-

[a]The reference of values palm oil, Jatropa oil, the US biodiesel and EU biodiesel standards are from Hoekman, 2012.

Table 3. Comparison of biodiesel properties from cultured oleaginous yeasts in molasses.

Yeast strains	Viscosity (mm²/s)	Specific gravity	Cloud point (°C)	Cetane number	Iodine number	HHV (MJ/kg)
Rhodosporidium toruloides TISTR 5123	5.21	0.8726	19.99	62.88	12.71	38.53
Rhodosporidium toruloides TISTR 5154	5.01	0.8743	15.86	60.81	35.71	39.08
Rhodosporidium toruloides TISTR 5149	5.21	0.8726	19.99	62.88	12.71	38.53
Yarrowia lipolytica TISTR 5054	4.61	0.8778	7.36	56.57	83.07	40.20
Yarrowia lipolytica TISTR 5151	5.21	0.8726	19.99	62.88	12.71	38.53
Yarrowia lipolytica TISTR 5621	4.98	0.8745	15.31	60.54	38.80	39.15
Rhodotorula glutinis TISTR 5159	4.93	0.8750	14.16	59.97	45.17	39.30
Rhodotorula graminis TISTR 5124	5.04	0.8741	16.45	61.11	32.42	39.00
Palm oil[a]	4.61	0.8730	14.0	61.9	54.0	40.6
Jatropa oil[a]	4.48	0.8789	4.67	55.23	98.20	40.55
	(4.75)	(0.876)	(5)	(55.7)	(109.5)	(40.7)
US biodiesel standard ASTM D6751[a]	1.9-6.0	-	-	47 min	-	-
EU Biodiesel standard EN 14214[a]	3.5-5.0	0.86-0.9	-	51 min	120 max	-

[a]The reference of values palm oil, Jatropa oil, the US biodiesel and EU biodiesel standards are from Hoekman, 2012.

Table 4. Comparison of biodiesel properties from cultured oleaginous yeasts in glycerol.

Yeast strains	Viscosity (mm²/s)	Specific gravity	Cloud point (°C)	Cetane number	Iodine number	HHV (MJ/kg)
Rhodosporidium toruloides TISTR 5123	5.21	0.8726	19.99	62.88	12.71	38.53
Rhodosporidium toruloides TISTR 5154	5.15	0.8731	18.73	62.24	19.77	38.70
Rhodosporidium toruloides TISTR 5149	5.21	0.8726	19.99	62.88	12.71	38.53
Yarrowia lipolytica TISTR 5054	5.21	0.8726	19.99	62.88	12.71	38.53
Yarrowia lipolytica TISTR 5151	5.00	0.8744	15.63	60.69	37.04	39.11
Yarrowia lipolytica TISTR 5621	4.96	0.8748	14.68	60.22	42.30	39.23
Rhodotorula glutinis TISTR 5159	5.21	0.8726	19.99	62.88	12.71	38.53
Rhodotorula graminis TISTR 5124	5.21	0.8726	19.99	62.88	12.71	38.53
Palm oil[a]	4.61	0.8730	14.0	61.9	54.0	40.6
Jatropa oil[a]	4.48	0.8789	4.67	55.23	98.20	40.55
	(4.75)	(0.876)	(5)	(55.7)	(109.5)	(40.7)
US biodiesel standard ASTM D6751[a]	1.9-6.0	-	-	47 min	-	-
EU Biodiesel standard EN 14214[a]	3.5-5.0	0.86-0.9	-	51 min	120 max	-

[a]The reference of values palm oil, Jatropa oil, the US biodiesel and EU biodiesel standards are from Hoekman, 2012.

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