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Production of second-generation biodiesel using low-quality date fruits

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ABSTRACT

This study focused on the valorization of the date syrup obtained from low-quality date fruits to be used as a low-cost alternative medium for producing single cell oil (SCO) by *Rhodotorula glutinis* PTCC5256, which could further be converted into biodiesel. The higher C/N ratio of date syrup (C/N 70) led to restricting the formation of cell biomass and enhancing the biosynthesis of SCO. The maximal cell biomass and lipid productivities were obtained 72 mg/L/h and 17 mg/L/h by C/N ratios of 20 and 70, respectively. Although the obtained biodiesel met the international standards for cold filter plugging point (4.92 °C), iodine value (87.22 g I_2/100 g oil), cetane number (52.26), higher heating value (40.19 MJ/kg), cloud point (6.29 °C), pour point (0.00 °C), density (878 kg/m^3), kinematic viscosity (4.30 mm^2/s) and oxidation stability (7.87 h), its weak cold-flow properties might limit its application in cold areas in comparison with diesel fuel.

1. Introduction

Biodiesel as a promising fuel for the future is a mixture of mono-alkyl esters of long-chain fatty acids like fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs), mainly provided *via* the transesterification of oils and short-chain alcohols with proper catalysts [1]. Having considerable advantages such as biodegradability, low exhalation of SO_x, CO_x, organic pollutants and chemical compounds, as well as its similar attributes to the petroleum-derived diesel, biodiesel has boosted notable world-wide attention as a renewable and environment-friendly energy source [2,3]. The selection of feedstock to manufacture biodiesel relies on the number of factors, including weather conditions, types of agricultural practices and products and economic aspects of its production; For instance, soybean oil is a predominant product in the United States (US) because of the environmental conditions. Biodiesel production should involve using cheap feedstocks since it is estimated that up to 75 % of the overall biodiesel generation costs result from the choice of raw material [4]. Microorganisms as an alternative to agricultural and animal sources have received increasing attention for the production of oils. They have developed a new way of reducing the high cost of biodiesel production since the methods concentrating on plant oils or animal fats require energy, acreage, feed and a proportionately long period of time for sufficient production [1,5].

Microbial oil which otherwise referred to as single cell oil (SCO) is intracellularly stored lipid, including triacylglycerol (TAG). SCO which is regarded as the edible oil can be attainable by yeasts, fungi and algae; merely a few bacteria reserve much extractable edible oil. All living microorganisms biosynthesize structural lipids (*e.g.*, membrane construction), however only a few microorganisms, the oleaginous ones, are able to generate lipid content more than 20 % of their dry biomass weight. The lipid begins to accumulate in the cell cytosol during the stationary growth phase as the cells are faced with nutrient constraints (*e.g.*, nitrogen or phosphor) and simultaneously with a surplus carbon source [6,7].

SCO production should involve utilizing low-cost substrates to limit the overall expenditure of fermentative processes. In other words, an efficient SCO-productive process promotes an economic system that makes use of inexpensive materials, whereas it gives a high-product yield. Besides, employing low-value bio-waste or wastewater for SCO production contributes to excusing the environment from highly contaminated wastes [8]. Various carbon sources ranging from zero cost raw materials (*e.g.*, livestock wastewater) to byproducts or wastes from agro-industrial processes (*e.g.*, sugarcane molasses) have been considered for the sustainable production of SCOs through fermentation, in recent decades [9,10]. Date syrup obtained from low-quality date fruits

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can be examined as a low-cost alternative medium for the growth of oleaginous microorganisms and SCO production.

Date palm (*Phoenix dactylifera* L.) plays an important role as a profitable product, which enables rural people in Middle Eastern and several Persian Gulf countries to improve their food safety, nutrition and income. While date palm has been become a primary commercial crop in the producing countries by exerting advanced technological strategies, date palm processing activities have not been developed at a similar rate. Besides, due to heavy rains, stormy conditions and degradation by birds and insects, a considerable amount of the harvested dates are damaged [11]. Comprising high concentrations of sugars, date syrup obtained from the low-quality date fruits could be considered for expanding fermentation sectors and biodiesel feedstocks. Low-cost substrates derived from date fruits are scarcely utilized for fermentation purposes. Elsanhoty et al. reported an increase in producing carotenoid with date syrup by *Lactobacillus plantarum* QS3B20 [12]. Also, in our recent studies, date syrup obtained from the low-quality date fruits was applied for the production of useful biomaterials using fermentative processes [13,14].

Several studies have evaluated the preparation of different types of biofuels (*e.g.*, bioethanol and butanol) by employing date palm fruits [15]. This study focused on the development of a process to use the date syrup obtained from low-quality date fruits as a low-cost alternative medium for producing SCO by oleaginous yeast *Rhodotorula glutinis* PTCC5256. Afterwards, the SCOs were implemented as the feedstock to produce lipid-based biofuel, and some characteristics of the generated biofuel were investigated.

2. Materials and methods

2.1. Strain and culture conditions

The oleaginous yeast *R. glutinis* PTCC5256 was obtained from the Persian Type Culture Collection (PTCC) and utilized for the production of SCO. The strain was maintained on yeast and malt extract (YM) agar slant enriched with 10 g/L dextrose, 3 g/L malt extract, 3 g/L yeast extract, 5 g/L peptone and 20 g/L agar at 4 ℃ until it was used. Some colonies from the agar slant were transmitted to 500 mL flasks comprising 100 mL of YM broth medium. The flasks were then incubated at 30 ℃ and 200 rpm for 24 h so as to prepare the seed culture.

2.2. Preparation and composition of date syrup

Low-quality date palms were collected from Jahrom, Fars, Iran. Stone-free date palm fruits were steeped in warm distilled water (50 ℃) for 30 min. Then the soaked fruits were thoroughly blended for 2 min at high speed by an electric drill (Ronix, Model 2106C, China) attached to the double-edged auger. The homogenized extract was filtered through a double-layer cheesecloth, and the residue was rinsed with hot water (85 °C). °Brix (total soluble solids) and pH of resultant syrup were fixed to 10 and 5.5, respectively, and thereafter each medium was sterilized by autoclaving at 121 °C for 15 min. For excluding sludge, the extracted syrup was centrifuged (SORVALL, RC-5; USA) at 5000×g for 15 min in sterile conditions [13].

The composition of date syrup was quantitatively determined according to the AOAC methods [16]: °Brix, nitrogen, total sugar, potassium and sodium by flame photometric, as well as magnesium, calcium, zinc, iron, copper and manganese by atomic absorption.

2.3. Fermentation in bioreactor

The batch fermentation processes were accomplished in a 5-liter-capacity bioreactor (Infors HT, Minifors, Switzerland) with a working volume of 3.5 L. Date syrup medium was inoculated with 1 % (v/v) of subculture and thereafter incubated for 96 h. During the fermentation processes, the pH level was maintained at 5.5 by automatically feeding 1.0 N sodium hydroxide solution into the bioreactor. The bioreactor was run at 30 ℃, and agitation speed during the process was set at 200 rpm. For evaluating the effect of different carbon/nitrogen (C/N) ratios of date syrup on biomass and lipid production by the oleaginous yeast *R. glutinis* PTCC5256, different C/N ratios of 20, 45 and 70 were implemented. The fermentation processes were carried out using native date syrup (C/N ratio of 45), supplemented date syrup with D-glucose (C/N ratio of 70) and supplemented date syrup with $(NH_4)_2SO_4$ (C/N ratio of 20).

The microbial growth was scrutinized by measuring the broth optical density at a wavelength of 600 nm. A common way of cell dry weight determination was practiced for biomass investigation. Briefly, a 10-ml specimen was poured into pre-weighed tubes and centrifuged at 8000 × g for 10 min, and then the pellets were dried at 105 ℃ in hot air oven to get constant weight.

2.4. Ultrasound-assisted extraction

Ultrasound-assisted extraction was utilized for extracting lipids of freeze-dried cells of *R. glutinis* produced in the submerged fermentation processes. Low-frequency high power ultrasonication (20 kHz, 150 W) extraction with a total operation time of 2 min, was carried out in an ultrasonic system (Bandelin, Germany) attached to a thermostatic water bath to forestall overheating. The extraction was fulfilled with the standard solvent mixture of chloroform and methanol (2:1 v/v) [17]. After extraction, the biomass was segregated from the solvent which was later eliminated from the procured supernatant by evaporation under vacuum. The steps of providing and extracting SCOs are shown in Fig. 1.

2.5. Transesterification

The approach for transforming the obtained crude lipid into FAME involved esterification following by hydrolysis of the lipids. The extracted SCOs solved in hexane and transesterified to biodiesel by base catalysis with 2 N KOH dissolved in methanol.

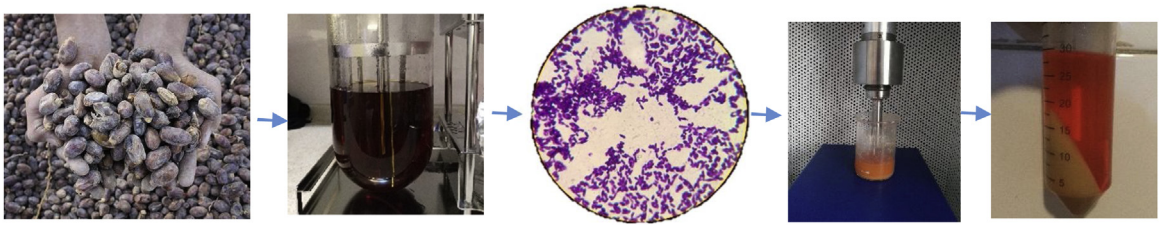

Fig. 1. Steps of producing and extracting SCO from R. glutinis PTCC5256 grown on date syrup obtained from low-quality date fruits.

Afterwards, the FAME profile was analyzed using the gas chromatograph (Thermo Finnigan, USA) which was equipped with a split/splitless injector, a fused silica CP-Sil 88 capillary column (length 100 m ×0.25 mm inner diameter with 0.25 μm film thickness) and a flame ionization detector (FID). The injector temperature of 250 °C was implemented for the injection of the 1 μl samples. The column temperature was scheduled to be between 140 and 240 °C at a pace of 3.2 °C/min. Nitrogen was exerted as the carrier gas, and the FID temperature was maintained at 260 °C during the investigation. FAMEs were characterized according to their retention times in comparison with the standard.

2.6. Determination of biodiesel fuel properties

Different physicochemical attributes of biodiesel *viz.*, degree of unsaturation (DU), long chain saturated factor (LCSF), cold filter plugging point (CFPP), saponification value (SV), iodine value (IV), cetane number (CN), higher heating value (HHV), cloud point (CP), pour point (PP), density (ρ), kinematic viscosity (ν_{mix}) and oxidation stability (OS), were determined for transesterified SCOs of the yeast strain PTCC5256. The proposed models which have been developed to predict the fuel properties of biodiesel are given as the following equations [18,19]:

$$DU = \%MUFA + (2 \times \%PUFA) \quad (1)$$

$$LCSF = (0.1 \times C16:0) + (0.5 \times C18:0) + (1 \times C20:0) + (1.5 \times C22:0) + (2 \times C24:0) \quad (2)$$

$$CFPP = (3.417 \times LCSF) - 16.477 \quad (3)$$

$$SV = \sum (560 \times A_i)/MW_i \quad (4)$$

$$IV = \sum (254 \times DB \times A_i)/MW_i \quad (5)$$

$$CN = 46.3 + (5458/SV) - (0.255 \times IV) \quad (6)$$

$$HHV = 49.43 - [0.041(SV) + 0.015(IV)] \quad (7)$$

$$CP = (0.526 \times C16:0) - 4.992 \quad (8)$$

$$PP = (0.571 \times C16:0) - 12.24 \quad (9)$$

$$\rho = \sum c_i \rho_i \quad (10)$$

$$\nu_{mix} = \sum A_c \times \nu_c \quad (11)$$

$$OS = 117.9295/X + 2.5905 \quad (12)$$

where A_i is percentage of the ith component in the mixture, MW_i is the molecular mass of each FAME, DB is the number of double bonds, c_i is the concentration (mass fraction) of the ith component, A_c is the relative amount (%) of the individual FAME component in the mixture, and X is is the content of linoleic and linolenic acids (wt %).

2.7. Statistical analysis

The mean values of samples were statistically analyzed *via* SPSS software (version 22.0). They were compared by applying one-way ANOVA and Duncan assay to disclose any significant differences among parameters and variables.

3. Results and discussion

3.1. SCO production by R. glutinis using date syrup

About10 liters of the date syrup (°Brix 10) was obtained from each kilogram of the initial low-quality date fruits. The ingredients of the utilized date syrup were quantitatively assayed, and the results were compiled in Table 1. The native date syrup was comprised of 8% total sugar and 0.7 % protein; since the predominant sugar in date palm and its by-product is the invert sugar [20], C/N ratio of the date syrup medium was estimated to be 45. Besides, the results verified that the date syrup contains adequate nutrients in large amounts to be appropriate for the growth of microorganisms. Date syrup as the foremost by-product of date palm processing, is recognized as the potential raw material to be utilized for the production of value-added microbial products using fermentation processes [12,13]. The attendance of 23 kinds of amino acids and at least six vitamins including vitamins C, B1, B2, A and niacin, makes date palms as an ideal feed which supplies a broad range of crucial nutrients for microbial growth and biosynthesis [21]. This section of our study centered on the valorization and biologic conversion of this substrate for the production of biomass and SOCs.

Although the application of pilot-plant aqueous extraction, which was performed in this study, contributes to the extraction of the main water-soluble components, insoluble proteins and fibres mainly comprised of cellulose, hemicelluloses, lignin, hemicelluloses, would slightly enter into the extracted syrup. The presence of fibrous particles causes problems, particularly during downstream processes. The aggregation and settling velocity of the fibers were enhanced by heating that was implemented for sterilizing the substrate after extraction. These insoluble substances were then excluded by centrifugation in sterile states. The elimination of these fibrous particles leads to obtaining appropriate substrate for the fermentation process. Shafiei et al. reported an amelioration in the settling rate of the fibers using enzymatic hydrolysis, which contributed to fewer obstacles in fermentation processes [22].

As described earlier, in order to produce SCO, three batch fermentation processes with different C/N ratios of date syrup were accomplished, and the variations of cell biomass, lipid

Table 1
Experimental results of date syrup composition.

Value	Parameter
10	°Brix
4.35	Initial pH
8.30	Total sugar (%)
0.52	Protein (%)
79.03	Calcium (mg/l)
0.359	Zinc (mg/l)
0.168	Iron (mg/l)
0.107	Copper (mg/l)
0.136	Manganese (mg/l)
196	Magnesium (mg/l)
46.0	Sodium (mg/l)
45.5	Potassium (mg/l)

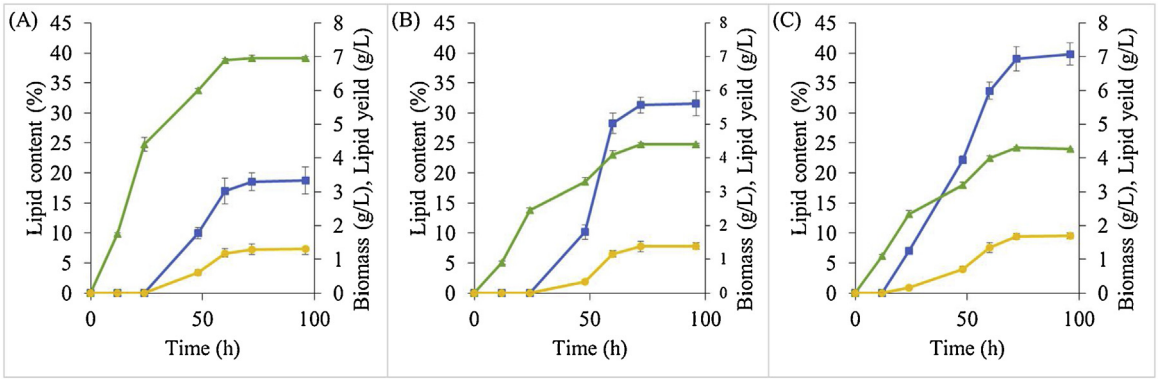

Fig. 2. Biomass (▲), lipid content (■) and lipid yield (●) obtained during batch cultivation of R. glutinis PTCC5256 in date syrup with (a) C/N ratio of 20, (b) C/N ratio of 45 and (c) C/N ratio of 70 within 96 h. The results were presented in mean ± SD, n = 3.

content and lipid yield during cultivation (96 h) of *R. glutinis* were investigated (See Fig. 2). *R. glutinis* started producing SCO after 24 h of incubation, which reached its maximum content after about 72 h. According to the results, generated in the media with distinct C/N ratios, the biomass was increased up to 60 h, and thereafter no further significant increase was detected. Also, the results revealed that while the biomass formation rate was different in the C/N 20 and C/N 45 media, the amounts of the SCO yield produced in those media were similar (~1.30 g/L). The maximal cell biomass and lipid productivities were achieved 0.072 g/L/h and 0.017 g/L/h with C/N 20 and C/N 70 media, respectively. Therefore, the higher C/N ratio of date syrup (C/N 70) led to restricting the generation of cell biomass and enhancing the biosynthesis of SCO. On the other hand, the lesser ratio of C/N (C/N 20) limited the biosynthesis of SCO (See Fig. 3). The presence of plentiful nitrogen at the beginning of the process would have been contributed to forming high amounts of biomass and limiting the generation of SCO.

After 96 h of culture, an increase in SCO production yield was observed for all three media in the range of 1.30–1.69 g/L. The characteristic parameters of the growth and lipid production by *R. glutinis* PTCC5256 cultivated in date syrup under aerobic condition are compiled in Table 2. A significant difference in the lipid contents between the three cultures should be occurred due to the nitrogen deficiency which arouses the cells to accumulate more lipids. However, there was a statistically significant difference between lipid contents of C/N 45 and C/N 70 media, no statistical difference was discerned between their lipid yields ($P < 0.05$), arisen from the generation of more biomass concentration in medium with C/N ratio of 45. The results revealed that the lipid production was significantly enhanced with increasing C/N ratio of date syrup between 20 and 70. Our data recommended that a design consisting a cultivation in the presence of abundant nitrogen to attain high biomass concentration followed by a cultivation with a high C/N ratio to produce high amounts of SCO would be most likely advantageous.

The results indicated that the cell biomass achieved in this study using the C/N 20 culture medium was significantly higher than the other ones ($P < 0.05$). In other words, the enrichment of date syrup growth medium with the nitrogen source (C/N 20 medium) incited the cells for more proliferation; nevertheless, it didn't induce the cells to accumulate more lipids in comparison with the other media. The maximum lipid production of 1.69 g/L was observed for the medium with C/N ratio of 70, which corresponded to the intracellular lipid content of 39.83 % cell dry weight. It is clearly suggested that the addition of D-glucose for the surfeit of carbon source in date syrup has enhanced the lipid accumulation rather than the biomass formation, and the high lipid content status significantly has increased the value of lipid

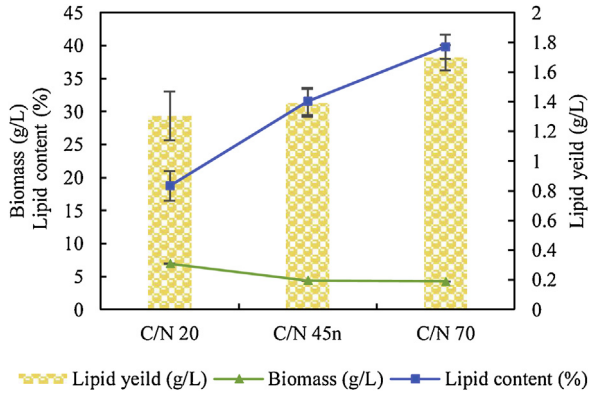

Fig. 3. The effect of C/N ratio on biomass concentration, lipid accumulation and lipid yield of *R. glutinis* PTCC5256 cultured in low-quality date syrup.

yield than the other media ($P < 0.05$). Indeed, a competition exists between the percentage of lipid content and the amount of biomass concentration, and the winner would be determined according to two factors, including the value of lipid yield and the economic perspectives of production.

It is clearly observed in the literature that the C/N ratio of substrate plays an incontrovertible role in the production of SCO by oleaginous microorganisms [23,24]. Braunwald et al. (2011) applied surface methodology (RSM) to optimize three factors for producing SCO by *R. glutinis* cultured in palm oil mill effluent. They reported that C/N ratio is the most influential parameter that affects the generation of SCO [25]. A C/N ratio of 20 is reported as a minimal state of SCO induction [26]. Similar to our results, several studies have been shown that SCO biosynthesis is more stimulated at higher C/N ratios [23,25]. In contrast to our results, some studies exhibit that lipid content increased by limiting the C/N ratio. In the study reported by Annamalai et al. (2018), waste office paper hydrolysate supplemented with nitrogen sources (C/N ratio of 80) was the most proper medium for high yield lipid production by *Cryptococcus curvatus* [27]. In another study, oleaginous alga *Chlorella protothecoides* was grown on low-nitrogen conditions, and it was reported that biomass formation was not influenced by restricting the C/N ratio, although lipid content increased [28].

When oleaginous organisms are grown with a nimiety of carbon source and a simultaneously restricted quantity of nitrogen source (high C/N ratio), they can accumulate a high concentration of lipids in the form of droplets of oil in their cytosol. Due to hydrophobicity enabling lipids to accumulate in high amounts in cells without alteration of cytosolic osmolarity, they could serve as the energy supplies to warrant the endurance of vital functions under

Table 2
Effect of C/N ratio on characteristic parameters of growth and lipid production by R. glutinis PTCC5256 cultivated in date syrup.

C/N Ratio	Biomass (g/L)	Biomass Productivity (g/L/h)	Lipid Content (%)	Lipid Yield (g/L)	Lipid Productivity (g/L/h)
C/N 20	6.95 ± 0.05^{a}	0.072	18.76 ± 2.25^{c}	1.30 ± 0.01^{b}	0.013
C/N 45	4.41 ± 0.02^{b}	0.045	31.56 ± 2.07^{b}	1.39 ± 0.09^{b}	0.014
C/N 70	4.26 ± 0.02^{c}	0.044	39.83 ± 1.85^{a}	1.69 ± 0.08^{a}	0.017

Values are representatives of mean ± S.D. (n = 3). Values followed by different superscripts in a column are significantly different ($P < 0.05$).

environmental stresses or in deficiency cases [29]. Two distinctive pathways involve in lipid accretion in oleaginous yeasts: de novo and ex novo lipid biosynthesis. De novo and ex novo pathways are systematically implemented when oleaginous microorganism respectively grows on hydrophilic and hydrophobic substrates. In the case of the de novo pathway, lipid formation as a secondary anabolic activity occurs following by nitrogen deficiency in a culture medium [6].

In this part of the research, we focused on the potential application of an economical alternative medium for producing SCO. About 10 L of the date syrup medium (°Brix 10) was afforded through each kilogram of the initial low-quality date palm fruits. The price for preparing each liter of the appropriated media, date syrup, is merely 0.05 % of the cost that should be allocated for formulating each liter of a synthetic culture medium like YM broth. Consequently, the application of date syrup medium rather than a synthetic medium contributed to 99 % saving in charges of medium provision. Date syrup found to be an adequate source for yeast growth and SCO production, while the addition of D-glucose for the surfeit of carbon source promoted the yield of SCO production. Indeed, the enrichment of waste media is not principally cost-effective, unless the SCO would be achieved with a justifiable yield, for instance, with optimizing production processes. The use of fed-batch cultivation mode has been considered in some studies, and both cell density and cellular lipid of oleaginous microorganism were dramatically enhanced [30]. Anyway, the improvement in SCO production using optimization of abiotic factors encounters various restrictions, and further researches should orient toward genetic modification of oleaginous microorganisms so as to biosynthesize and accumulate more SCOs.

The biosynthesis of SCO from different agro-industrial wastes is a promising trajectory toward the accomplishment of low-cost bioprocesses. Three widespread agro-industrial wastes including glycerol, orange peel extract and ricotta cheese whey were transformed into SCO by employing nine oleaginous fungi which belong to the *Aspergillus*, *Mucor*, *Mortierella* and *Cunninghamella* genera [31]. In another study, hydrolysate of cassava starch was used to be efficiently converted into SCO by culturing marine-derived yeast *R. mucilaginosa* TJY15a [32]. Also, Liu et al. reported an enhanced lipid production from undetoxified corncob hydrolysate by *R. glutinis* using a high cell density culture *via* two-step nitrogen feeding procedure [30]. Another study successfully investigated the production of SCO by *Yarrowia lipolytica* Po1g from detoxified sugarcane bagasse hydrolysate as an substitute carbon source [33].

3.2. Quality of biodiesel fuel

The obtained lipid from the strain PTCC5256 grown on the native date syrup was straightly transmethylated to achieve FAME or biodiesel. According to the results, the fatty acid composition of the produced SCOs primarily belongs to long-chain fatty acids with 16 and 18 carbon atoms (See Table 3). The total content of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in the transesterified lipid were found to be 29.34 %, 48.29 % and 22.34 %, respectively. Also, no proportion of PUFA comprising ≥ 4 double bonds, which are not favorable for biodiesel (1 % max, according to EN 14,214), was detected. According to Eq. (1) the DU which dramatically affects the kinematic viscosity and density was calculated to be 92.97. Besides, based on Eq. (2), LCSF was calculated to be 6.26. The comparison between the predominant fatty acids identified in this study and the fatty acids composition of the lipids produced from other low-cost substrates using oleaginous yeast *R. glutinis* inoculation is summarized in Table 4.

Table 3
Fatty acids profile of lipid derived from R. glutinis PTCC5256 grown on date syrup.

Distribution of fatty acids	%
Tetradecanoic acid (C14:0)	1.06 ± 0.47
Hexadecanoic acid (C16:0)	21.44 ± 2.17
Hexadec-9-enoic acid (C16:1)	1.55 ± 0.42
Octadecanoic acid (C18:0)	6.20 ± 0.28
(9Z) Octadec-9-enoic acid (C18:1n9c)	46.74 ± 2.45
cis, cis-912-octadecadienoic acid (C18:2n6c)	14.18 ± 1.50
all-cis-6,912-octadecatrienoic acid (C18:3n6)	8.16 ± 0.38
Icosanoic acid (C20:0)	0.27 ± 0.04
Docosanoic acid (C22:0)	0.00 ± 0.00
Tetracosanoic acid (C24:0)	0.38 ± 0.06
Total SFA	29.34
Total MUFA	48.29
Total PUFA	22.34

The comparison of profiles indicates some differences in the percentage of fatty acids, depending on the type of medium utilized. In other words, assuming that the SCO production in various media have been fundamentally applied in the same condition, the type of the utilized substrate has affected the metabolic pathway of lipid production; hence the fatty acid composition of the produced oils, like the percentage of oil content, has altered. In a similar study, various substrates *i.e.*, glucose, banana peel and sugarcane bagasse were employed for fungal growth and lipid production, and the results exhibited that both type and concentration of fatty acids were achieved relatively different depending on the medium used [37]. So, it can be inferred that in order to produce SCOs which could be further used as a desirable biodiesel feedstock, preliminary study on the modification of culture medium for providing suitable fatty acid profile, would be beneficial. In general, high-quality biodiesel requires both saturated and unsaturated FAMEs in optimal balance, though oleic acid is being recognized as optimal fatty acid for ameliorating the fuel features [23].

The fatty acid composition of lipid that is used as biodiesel feedstock fundamentally determines the quality and characteristics of biodiesel. The attendance of high levels of the long-chain MUFA promotes biodiesel quality as they are fluid at room temperature and could develop biodiesel flow attributes. A high percentage of PUFAs not only affects the oxidative stability and quality of lipid-based biodiesel during storage but also leads to increased viscosity which is unsuitable for biodiesel [38]. Consequently, since an ideal biodiesel is principally made from methyl esters of both SFA and MUFA with low PUFA, the SCO produced by *R. glutinis* PTCC5256 grown on date syrup could be considered as appropriate feedstock for being converted into biodiesel.

Table 4
Comparison of fatty acid profile of lipids accumulated by *R. glutinis* grown on various media.

C16:0	C18:0	C18:1	C18:2	C18:3	Carbon Source	Reference
16.01	21.86	18.05	15.91	1.76	Glycerol	[34]
13.30	5.10	55.50	20.20	6.30	Lignocellulosic biomass hydrolysate	[35]
29.60	10.10	37.90	22.90	1.90	Miscanthus hydrolysate	[36]
31.40	9.00	31.60	19.30	3.40	Wheat straw hydrolysates	[36]
33.10	3.25	46.90	14.30	0.00	Undetoxified corncob hydrolysate	[30]
20.37	10.33	47.88	7.31	0.85	Palm oil mill effluent	[25]
21.44	6.20	46.74	14.18	8.16	Low-quality date syrup	[This work]

Since the direct analysis of fuel characteristics of biodiesel is complicated, as well as the analysis requires a considerable amount of fuel sample [39], various prediction models and mathematical equations have been developed to anticipate fuel properties of lipid-based biodiesel from their FAMEs composition. In the current study, different physicochemical attributes of the produced biodiesel were determined based on Eqs. (1)–(12), and the results were compiled in Table 5.

The cloud point (CP), pour point (PP) and cold filter plugging point (CFPP) are regarded as the most important cold-flow properties of biodiesel fuels [42]. Cloud point and pour point of biodiesel were estimated to be 6.29 °C and 0.00 °C, respectively, which met the standard requirements imposed by ASTM. Cloud point is determined as the temperature wherein crystals start forming in biodiesel, whilst pour point is the temperature at which biodiesel won't flow anymore due to the formation of numerous agglomerated crystals [43]. Also, the cold filter plugging point of biodiesel (4.92 °C) lay within the specified range of EN14214 standard (−20 to 5 °C). The cold filter plugging point is defined as the minimum temperature at which a specific volume of biodiesel passes through a filter within 60 s as a vacuum of 2 kPa is applied to the system [43]. The weak cold-flow properties of the generated biodiesel limit its application in cold areas in comparison to diesel fuel. In general, possessing higher cloud and pour points than the conventional diesels, biodiesels exhibit lower cold-flow property, which is one of the most critical obstacles against their usage in cold regions [44].

Iodine value (IV) which is a rough estimate of the degree of unsaturation was predicted to be 87.22 g I_2/100 g oil. In order to examine the levels of stability to oxidation, iodine value was introduced in biodiesel standards. An optimal content of methyl esters in biodiesel is required to avoid solidification. However, a higher degree of unsaturation is not proper for biodiesel. The higher number of double bonds contributes to getting enhanced reactions with atmospheric oxygen and the generation of hydroperoxide, which can lead to acidification and polymerization [44,45]. Hence, the risk of deposits and filter plugging in the fuel system is raised [43]. The obtained biodiesel met the international standards for the oxidation stability (OS) (7.87 h).

Cetane number (CN) and higher heating value (HHV) of the generated biodiesel were determined to be 52.26 and 40.19 MJ/kg, respectively. Characterizing the combustion quality of diesel fuels, cetane number determines the ease of self-ignition of fuel injected into the combustion chamber [46]. Higher heating value is determined as the liberated heat from a unit fuel burned in oxygen if all products generated from combustion are allowed to cool down to 25 °C and the water vapour formed during ignition is condensed to a liquid [43]. Moreover, saponification value (SV) which relies on the molecular weight of fatty acids existing in FAMEs of biodiesel was anticipated to be 193.57 mg KOH/g oil. The values of cetane, iodine and higher heating were found to lie within the ranges of international biodiesel standards.

Density (ρ) and kinematic viscosity (ν_{mix}) of the generated biodiesel were predicted to be 878 kg/m^3 and 4.30 mm^2/s, respectively, which met the standard requirements. The density and viscosity are highly critical for biodiesel fuel as they play a prominent role in the quality of fuel atomization mechanism and air-fuel mixture ignition. Those crucially influence the quantity of injected fuel, injection timing and fuel spray pattern [18,43,47].

The current study focused on producing a type of biofuel generated based on the lipid which accumulated in oleaginous yeast *R. glutinis* PTCC5256 during growth on the date syrup achieved from low-quality date palm fruits. In other words, the second-generation biodiesel was produced from low-quality date fruits which are not considered as a food crop. Indeed, the preference for generating second-generation biofuels is due to that those do not normally utilize the food crops as feedstock. By contrast, first-generation biofuels are produced using feedstocks which can be used for food as well (*e.g.*, soybean oil, canola oil, and sunflower oil) [48]. To the best of our knowledge, this is the first work that shows obtaining biodiesel by employing date palm fruits. However, a few studies have been conducted on applying

Table 5
Comparison of the main fuel properties of biodiesel generated in this study with international biodiesel standards and diesel fuel.

Properties	Biodiesel	Biodiesel standards		Diesel fuel [40,41]
		EN14214	ASTM D6751	
FAME with ≥ 4 double bonds (%)	ND	1 max	NS	–
Linolenic acid content (%)	8.16	12 max	NS	–
DU	92.97	NS	NS	–
LCFS	6.26	NS	NS	–
CFPP (°C)	4.92	−20–5	NS	−12
SV (mg KOH/g oil)	193.57	NS	NS	–
IV (g I_2/100 g oil)	87.22	120 max	NS	–
CN	52.26	51 min	47–65	51
HHV (MJ/kg)	40.19	~35 min	NS	46.1
CP (°C)	6.29	NS	−3–15	−18
PP (°C)	0.00	NS	−5–10	−25
ρ (g/cm^3)	0.878	0.860–0.900	NS	0.825
ν_{mix} (mm^2/s)	4.30	3.50–5.00	1.90–6.00	2.50
OS (h)	7.87	6 min	3 min	–

ND: not detected, NS: not specified.

date seed (or stone) oil to study the production of biodiesel [44,49,50].

Amani et al. investigated the possibility of employing date seed oil as a low-cost feedstock for biodiesel generation and applied various theoretical models so as to predict and evaluate the biodiesel characteristics. According to their results, low viscosity and flash point, as well as high cetane number, were the most important advantages of the date seed-derived biodiesel [44]. In the study reported by Fadhil et al. extracted oils from date palm stones were utilized for the production of different types of biodiesels [51]. Another study successfully examined the production biodiesel in the attendance of heterogeneous metallic oxide catalyst by the use of date seed oil [50]. Also, in the study reported by Farooq et al., waste chicken egg shells were exerted as an efficient catalyst in order to generate biodiesel using date seed oil [49]. Those catalysts rendered reusability and substantial stability during trans-esterification. Recently, some promising studies have begun to accelerate the synthesis of biodiesel using ultrasound technology. Increasing the interfacial surface area between reactants (*i.e.*, oil and alcohol), ultrasound irradiation contributes to higher biodiesel yield within less time [52,53].

4. Conclusions

The production of SCOs for utilizing as an appropriate biodiesel feedstock should involve using low-cost substrates to minimize the overall expenditure of fermentative processes. In the current study, the cost of lipid production by the oleaginous yeast *R. glutinis* PTCC5256 dramatically decreased by employing date syrup obtained from low-quality date fruits as substrate. The produced lipid found to be a promising alternative feedstock for the production of second-generation biodiesel since the biodiesel characteristics lay within the imposed limits of the international standards. The study recommends that the production of SCO using date syrup might be promoted by optimizing the parameters associated with culture medium and cultivation mode. For instance, the production would be improved by accomplishing the partial detoxification of low-quality date syrup. Furthermore, the use of fed-batch cultivation mode would most probably enhance the formation of biomass and the yield of lipid produced. On the other hand, the genetic of oleaginous microorganism *R. glutinis* could be deliberately modified so as to rectify the fatty acids profile of the lipid which could further be used as a desirable biodiesel feedstock. Date syrup obtained from low-quality date fruits could be considered as a low-cost substrate for producing other microbial products in the future.

CRediT authorship contribution statement

Abouzar Ghasemi: Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Visualization. **Marzieh Moosavi-Nasab:** Validation, Formal analysis, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

All authors declare no conflicts of interest in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00480.

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