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Bioconversion of Some Agro-industrial By-products into Single Cell Oil Using *Candida albicans* NRRL Y-12983 and *Lipomyces starkeyi* NRRL Y-11557

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ABSTRACT

In view of an ever-increasing demand for oils and fats, there is an urgent need to develop non-conventional sources for its production, such as microbial sources. Single cell oil (SCO) production by strains of oleaginous yeasts i.e., *Candida albicans* NRRL Y-12983 and *Lipomyces starkeyi* NRRL Y-11557 grown on various agro-industrial by-products is a focus of current attention. The highest SCO productivity by both yeast strains was obtained at initial pH 6.0 and 25°C with agitation at 200 rpm after 72 h incubation. Sugar beet molasses and glucose syrup were the best inducers for SCO production by *C. albicans* NRRL Y-12983 and *L. starkeyi* NRRL Y-11557 (1.5 g L^{-1} and 1.4 g L^{-1} , respectively). Among nitrogen sources, corn gluten meal and protelan greatly induced SCO production by *C. albicans* NRRL Y-12983 and *L. starkeyi* NRRL Y-11557. (1.5 g L^{-1} and 1.6 g L^{-1}, respectively). Palmitic (C16:0) and oleic (C18:1) acids were produced in highest values (20.544 and 54.261 mg g^{-1} sample, respectively) by *C. albicans* at 25°C; their values being 23.988 and 37.408 mg g^{-1} sample by *L. starkeyi* at 15°C. Linoleic acid (C18:2) was produced in highest amount of 41.302 mg g^{-1} by *C. albicans* at 15°C and 39.073 mg g^{-1} by *L. starkeyi* at 20°C. It can be concluded that oleaginous yeasts is a very efficient and inexpensive sources for SCO production. The ease, with which they can be cultivated, has facilitated the large-scale production of the microbial oil.

Key words: Single cell oil, *Candida albicans*, *Lipomyces starkeyi*, agro-industrial by-products, fatty acids, growth temperature

INTRODUCTION

With the increasing demands for fats and oils for edible and industrial purposes, many efforts have been achieved to find new microbial sources other than the conventional plant and animal sources (Azeem *et al*., 1999). However, microbial oils were found to be more feasible and are comparable with the composition of conventional plant oils. Oleaginous yeasts are considered for the production of single cell oil (SCO), where the economics of SCO has become

more favorable when utilizing waste substrate as carbon and/or nitrogen source (Pan *et al*., 2009). The bioconversion of low cost agro-industrial wastes to SCO is considered an alternative way to decrease the cost of SCO production process with simultaneous recycling of these wastes. Many oleaginous yeast species accumulated Triacylglycerols (TAGs) from 20 to 70% of biomass under appropriate cultivation conditions. They include *Rhodosporidium toruloides*, *Cryptococcus albidus*, *Lipomyces starkeyi*, *Rhodotorula glutinis*, *Trichosporon pullulan* and *Yarrowia lipolytica* (Ratledge and Wynn, 2002; Daum *et al*., 2007; Kraisintu *et al*., 2010). In addition, the chemical structure of extracted lipids produced by *Cryptococcus curvatus* NRRL Y-1511 was linoleic acid (30.68%), oleic acid (22.66%) and palmitic acid (16.74%). The value of total saturated fatty acids was 41.96%, whereas, the value of total unsaturated fatty acids was 58.04% (El-Fadaly *et al*., 2009).

Lipids are stored in special organelles, i.e., lipid particles or lipid bodies, by which triacylglycerol and steryl esters (STEs) are the most storage lipids of eukaryotic cells, e.g., yeasts. Furthermore, TAG and STE form the core of the lipid particles; they are surrounded by phospholipids monolayer with small amount of proteins embedded (Sorger *et al*., 2004). Polyunsaturated Fatty Acids (PUFAs), especially those with 20 carbon atoms, can possess medical effects, e.g., preventing blood platelets aggregation (Lindberg and Molin, 1993) as well as lowering of plasma cholesterol (Sugano *et al*., 1986). PUFAs can naturally be found in low levels in porcine liver as arachidonic acid (AA, C20: 4W6) and in fish oils as eicosapentaenoic acid (EPA, C20: 5W3) and docosahexaenoic acid (C22: 6W3). There are also fungal strains able to produce Arachidonic Acid (AA) and Eicosapentaenoic Acid (EPA), e.g., the genus *Mortierella* (Jang *et al*., 2005; Hou, 2008).

In the present study, we aimed to reduce the production cost of SCO by using agro-industrial by-products as carbon and nitrogen sources, to evaluate the best conditions for production of SCO by *Candida albicans* NRRL Y-12983 and *Lipomyces starkeyi* NRRL Y-11557 and for quantitative determination of the microbial oil composition (fatty acids obtained from the yeasts under study) as affected by growth temperature.

MATERIALS AND METHODS

Yeast strains and cultivation media: The yeast strains used in this investigation were *Candida albicans* NRRL Y-12983 and *Lipomyces starkeyi* NRRL Y-11557. The two yeast strains were provided from the Agricultural Research Service (ARS) culture collection, Northern Regional Research Laboratory (NRRL), Peoria, Illinois, USA. The cultivation medium of Lindberg and Molin (1993) was used for fermentation and cultivation of the yeast strains. Yeast extract malt broth (YMB) medium was used for inoculum preparation and maintenance of the used yeast strains.

By-products used: Sugar beet molasses (SBM) that obtained from sugar beet factory of Belkas, Dakahlia Governorate, Egypt was used as a sole carbon source. This by-product contains about 48% total sugars before purification that became 23.3% after purification. Corn gluten meal obtained from starch and glucose factory as a by-product containing 2.22% nitrogen was used as a sole nitrogen source. Each of these wastes was replaced with the other source in the cultivation medium of Lindberg and Molin (1993) in the same ratio.

Preparation of the industrial by-products: Sample of sugar beet molasses (SBM) was prepared as carbon source by diluting with water in an equal volume using the method of Pandey and Agarwal (1993) with little modification. H_2SO_4 solution was used to reduce the pH

value to reach 3.0. Sample was boiled at 100°C for 1 h then maintained at room temperature for 24 h, centrifuged at 3000 rpm and filtered through filter paper. Filtrated solution was used to determine the total sugars to be used as a sole carbon source. Nitrogenous by-product namely corn gluten meal, was oven dried at 105°C for 2 h and milled. About 7.5 g of waste was add to 100 mL of 1.5% H_2SO_4 and autoclaved for 45 min then filtered. The obtained supernatant was attained at pH 6, being used as a sole nitrogen source.

Inocula preparation and fermentation: The inocula used in the experiments were prepared in Erlenmeyer flasks using YMB medium. The cultivation conditions were carried out at 28°C; pH 6; 72 h and shake speed was 200 rpm. Fermentation was carried out in 250 mL capacity Erlenmeyer flasks containing 100 mL of the previously mentioned fermentation medium and initial pH was adjusted to 6.0 using a pH meter before autoclaving at 121°C for 20 min. The experimental flasks were then inoculated with 3% v/v (5×10^5 cfu mL^{-1}) of cells suspension of the examined yeast prepared in sterile distilled water. The culture was incubated at the growth temperature on a rotary shaker at 200 rpm for the required fermentation period using LAB-line instrument, Inc., plaza, Mel Rose, Park, ILL. 60160.

Six agro-industrial by-products as carbon sources namely sugar beet molasses, sugar cane molasses, potato peels, tomato peels, glucose syrup and squash peels were individually added to the basal cultivation medium at a concentration of 10% to study the effect of each carbon source on the production of microbial oil.

Determination of yeast dry weight (g L^{-1}): Yeast dry weight (single cell protein) determination was performed by harvesting the fermentation culture, centrifuged at 5000 rpm, washed twice with distilled water and dried at 60°C to constant weight (Granger *et al*., 1993). The growth yield efficiency (economic coefficient) was calculated according to the following equation:

$$\text{Growth yield efficiency} = \frac{\text{Cell dry weight } (\text{g L}^{-1})}{\text{Sugar consumed } (\text{g L}^{-1})}\times 100$$

The productivity of oil produced (conversion coefficient) was also calculated according to the following equation:

$$\text{Single cell oil productivity} = \frac{\text{Single cell oil weight } (\text{g L}^{-1})}{\text{Cell dry weight } (\text{g L}^{-1})}\times 100$$

Determination of total sugars: Total sugars were determined according to the method of Herbert *et al*. (1971).

Single cell oil extraction: Single cell oil extraction was carried out according to the method of Granger *et al*. (1992). The single cell oil yield efficiency was calculated according to the following equation:

$$\text{Single cell oil yield efficiency} = \frac{\text{Single cell oil weight } (\text{g L}^{-1})}{\text{Sugar consumed } (\text{g L}^{-1})}\times 100$$

Extraction of fatty acids: The method of extraction for fatty acids determination of obtained single cell oil was carried out according to the method recommended by Radwan (1978).

Gas chromatographic conditions: Sample was injected into a Shimadzu Model GC-8A gas chromatograph equipped with a flame ionization detector (FID) and a 2.5 M×3 mm glass column under the following conditions, 5% DEGS coated on 80-100 mesh with chromosorb WHP. The flame ionization detector temperature was 270°C and the column set was temperature programmed from 150 to 180°C at 2°C min^{-1}. The carrier gas was nitrogen, with a flow rate of 20 mL min^{-1}; hydrogen flow rate was 75 mL min^{-1} and chart speed 2.5 mm min^{-1}. The hydrogen and air flow-rates were 75 and 0.5 mL min^{-1}, respectively. FID sensitivity was 16×10^2. Peaks of fatty acids were identified by chromatography with the standards and relative retention times (Radwan, 1978).

RESULTS AND DISCUSSION

Single cell oil production as affected by carbon source: A laboratory batch fermentation process has been designed to study the individual effect of six agro-industrial by-products as carbon sources on single cell oil (SCO) production by *Candida albicans* NRRL Y-12983 and *Lipomyces starkeyi* NRRL Y-11557. Data as shown in Table 1 indicate that the final pH values were appreciably changed in the range of 5.0 to 6.6 during the fermentation process. The highest values of the consumed sugars (45.6 and 40.4 g L^{-1}) and cell weights (7.4 and 8.5 g L^{-1}) were obtained by fermenting glucose syrup by *C. albicans* NRRL Y-12983 and sugar beet molasses by *L. starkeyi* NRRL Y-11557. Interestingly, the bioconversion of glucose syrup (*C. albicans*) and sugar beet molasses (*L. starkeyi*) into SCO was positively correlated with obtained cell weights (g L^{-1}). Further, the maximum productivity (dp/pt) reached up to 0.0310 and 0.029 g L^{-1} h^{-1} by *C. albicans* on sugar beet molasses and *L. starkeyi* on glucose syrup, respectively. These results are inconsistent with the finding of Evans and Ratledge (1983), who stated that, in batch culture of *Candida curvata* D, lactose supported the best biomass production whereas; xylose was the best for lipids production. Likewise, Li *et al.* (2007) pointed out that lipid accumulation in yeast requires sugar-rich medium or similar components such as glycerol and polysaccharides. The accumulation of lipids in *Lipomyces starkeyi* depends upon C/N ratio (Angerbauer *et al.*, 2008).

Table 1: Single cell oil and single cell protein production on some industrial by-products as carbon sources

Yeast strain	Carbon sources	Final pH	Consumed sugar(g L^{-1})	Cell weight (g L^{-1})	Oil weight (g L^{-1})	Growth yield efficiency (%)	Oil yield efficiency(%)	Oil (%)	dx/dt	dp/dt
Candida	Sugar cane molasses	5.0	40.2	6.5	1.1	16.16	2.73	16.92	0.1350	0.0229
albicans	Sugar beet molasses	5.2	42.4	7.4	1.5	17.45	3.53	20.27	0.1540	0.0310
NRRL Y-	Glucose syrup	5.8	45.6	7.2	1.2	15.78	2.63	16.66	0.1500	0.0250
129830	Potato peels	6.3	32.8	5.4	0.8	16.46	2.43	14.81	0.1120	0.0166
	Tomato peels	6.2	34.7	5.8	0.9	16.71	2.59	15.52	0.1200	0.0187
	Squash peels	6.4	38.2	5.2	0.6	13.61	1.57	11.54	0.1080	0.0125
Lipomyces	Sugar cane molasses	5.2	38.3	7.4	1.2	19.32	3.13	16.22	0.1540	0.0250
starkeyi	Sugar beet molasses	5.6	40.4	8.2	1.2	20.29	2.97	14.63	0.1700	0.0250
NRRL Y-	Glucose syrup	6.2	35.6	8.5	1.4	23.87	3.93	16.47	0.1770	0.0290
11557	Potato peels	6.5	33.1	6.6	0.8	19.93	2.41	12.12	0.1370	0.0166
	Tomato peels	6.6	30.8	5.4	0.6	17.53	1.94	11.11	0.1120	0.0125
	Squash peels	6.6	29.9	4.9	0.6	16.38	2.05	12.24	0.1020	0.0125

dx: Δ biomass, dt: Δ time, dp: Δ product

Table 2: Single cell oil and single cell protein production on some by-products as nitrogen sources

Yeast strain	Nitrogen source	Final pH	Consumed sugar (g L^{-1})	Cell weight (g L^{-1})	Oil weight (g L^{-1})	Growth yield efficiency (%)	Oil yield efficiency (%)	Oil (%)	dx/dt	dp/dt
Candida	Corn gluten meal	5.6	69.2	6.5	1.5	9.39	2.16	23.07	0.1350	0.0312
albicans	Corn steep liquor	6.2	52.4	4.2	0.8	8.01	1.53	19.04	0.0875	0.0166
NRRL Y-	Protelan	6.5	70.6	6.4	1.0	9.06	1.42	15.62	0.1333	0.0208
12983	NH_4Cl	6.0	55.9	4.9	0.8	8.76	1.43	16.32	0.0102	0.0167
Lipomyces	Corn gluten meal	5.4	66.7	7.4	1.2	11.09	1.79	16.22	0.1540	0.0250
starkeyi	Corn steep liquor	5.8	58.2	6.6	0.9	11.34	1.55	13.64	0.1370	0.0187
NRRL Y-	Protelan	6.6	74.3	8.6	1.6	11.57	2.15	18.60	0.1790	0.0333
11557	NH_4Cl	5.8	60.4	6.5	0.8	10.76	1.32	12.30	0.1350	0.0166

dx: Δ biomass, dt: Δ time, dp: Δ product

Similarly, beet molasses was the most inducer for accumulation of lipids in *Candida curvata* D (Bednarski *et al*., 1986). Whereas; glucose was found to be the best for lipid accumulation by *M. alpina* CBS 343.66 (Lindberg and Molin, 1993).

Single cell oil production as affected by nitrogen source: Three agro-industrial by-products i.e., corn gluten meal, corn steep liquor and protelan were used, individually, as nitrogen source in the fermentation medium of *C. albicans* NRRL Y-12983 and *L. starkeyi* NRRL Y-11557. Data (Table 2) indicate that the final pH values of the fermentation medium changed according to the yeast strain and the nature of nitrogen source. The corn gluten meal was superior to other nitrogen sources, in which the yeast cell and single cell oil weights being 6.5 and 1.5 g L^{-1} by *C. albicans*, respectively. However, the highest value of consumed sugar (74.3 g L^{-1}), cell weight (8.6 g L^{-1}) and oil weight (1.6 g L^{-1}) were obtained during the fermentation of protelan by *L. starkeyi*. Vega *et al*. (1988) found that yeast extract has effect on the accumulation of lipids in the *Apiotrichum curvatum* ATCC 20509, whereas the asparagine was the best inducer for lipids accumulation. Although, the mixture of KNO_3 and yeast extract at 2: 1 (w/w) was the best nitrogen source for lipid and total polyunsaturated fatty acids production by *Mortierella alpina* (Jang *et al*., 2005). Previous studies illustrated that a medium with low content of nitrogen, the activity of Nicotinamide Adenine Dinucleotide Isocitrate Dehydrogenase (NAD-IDH) decreases or even disappears from the mitochondria of oleaginous yeasts, then Tricarboxylic Acid Cycle (TCA) is repressed, metabolism pathway altered, protein synthesis stopped and lipid accumulation activated (Palmieri *at al*., 1996; Pan *et al*., 2009). In addition, organic nitrogenous compounds are good for lipid accumulation, but not for cell growth (Huang *et al*., 1998).

Effect of initial pH on single cell oil production: The growth yield efficiency, oil yield efficiency, as well as, cell and oil weights of *C. albicans* and *L. starkeyi* as response to initial pH values of fermentation medium was determined (Table 3). Data reveal that the initial pH 6.0 was the optimum for higher yield of growth (15.53%) and oil efficiency (3.1%) by *C. albicans*. Meanwhile, the highest oil yield efficiency (3.21%) was obtained at initial pH 5.5 by *L. starkeyi*. The SCO productivity showed to be the highest at initial pH of 6.0 by *C. albicans* and *L. starkeyi*. The oil percentage (20.0%) was found to be the best at initial pH 6.0 by *C. albicans*, whereas this value (20.18%) was found at initial pH 5.5 by *L. starkeyi*. In earlier study, the pH value of 6.5 was

Table 3: Effect of initial pH values on single cell oil and single cell protein production

Yeast strain	Initial pH value	Final pH	Consumed sugar (g L^{-1})	Cell weight (g L^{-1})	Oil weight (g L^{-1})	Growth yield efficiency (%)	Oil yield efficiency (%)	Oil (%)	dx/dt	dp/dt
Candida	4.0	4.4	65.1	8.5	1.3	13.05	1.99	15.29	0.1770	0.0270
albicans	4.5	4.8	64.3	8.8	1.6	13.68	2.48	18.18	0.1833	0.0333
NRRL Y-	5.0	5.2	66.6	9.3	1.7	13.96	2.55	18.27	0.1937	0.0354
12983	5.5	5.8	68.2	10.2	1.7	14.95	2.49	16.66	0.2125	0.0354
	6.0	5.8	64.4	10.0	2.0	15.53	3.10	20.00	0.2080	0.0416
Lipomyces	4.0	4.6	60.5	9.6	1.6	15.86	2.64	16.66	0.2000	0.0333
starkeyi	4.5	4.6	64.2	9.9	1.9	15.42	2.95	19.19	0.2060	0.0395
NRRL Y-	5.0	5.3	65.6	10.6	2.1	16.15	3.20	19.81	0.2208	0.0437
11557	5.5	5.9	68.5	10.9	2.2	15.91	3.21	20.18	0.2270	0.0458
	6.0	6.4	72.8	12.1	2.3	16.62	3.16	19.00	0.2520	0.0479

dx:Δ biomass, dt: Δ time, dp: Δ product

Table 4: Single cell oil and single cell protein production as affected by incubation period

Yeast strain	Incubation interval (h)	Final pH	Consumed sugar (g L^{-1})	Cell weight (g L^{-1})	Oil weight (g L^{-1})	Growth yield efficiency (%)	Oil yield efficiency (%)	Oil (%)	dx/dt	dp/dt
Candida	24	4.8	66.2	8.3	1.2	12.54	1.81	14.45	0.3450	0.0500
albicans	48	5.2	68.3	8.8	1.4	12.88	2.05	15.90	0.1833	0.0291
NRRL Y-	72	5.6	64.5	8.0	1.6	12.40	2.48	20.00	0.1111	0.0222
12983	96	5.0	61.2	7.8	1.2	12.74	1.96	15.38	0.0813	0.0125
Lipomyces	24	5.1	55.4	10.2	1.5	18.41	2.71	14.70	0.4250	0.0625
starkeyi	48	5.3	58.6	11.3	1.8	19.28	3.07	15.93	0.2354	0.0375
NRRL Y-	72	5.6	64.8	12.4	2.1	19.13	3.24	16.93	0.1722	0.0292
11557	96	5.2	59.7	12.2	1.8	20.43	3.01	14.75	0.1270	0.0187

dx: Δ biomass, dt: Δ time, dp: Δ product

the best for growth and high production of polyunsaturated fatty acids (Lindberg and Molin, 1993), however, the best pH for biomass and lipid production together with high proportion of polyunsaturated fatty acids was around 6 (Bajpai *et al*., 1991; Yamada *et al*., 1987).

Single cell oil production as affected by incubation period: As shown in Table 4, data indicate that cell weight (8.8 g L^{-1}) and growth yield efficiency (12.88%) were produced in highest amounts by *C. albicans* after 48 h incubation period. However, the highest yield of oil percentage (20.0%) was obtained after 72 h. Similarly, the highest yield of oil percentage (16.93%) by *L. starkeyi* has been obtained after 72 h. Contrarily; the highest SCO productivity has been obtained by *C. albicans* and *L. starkeyi* after 24 h. Previous study showed that production of polyunsaturated fatty acids gradually decreased in prolonged cultivation due to cell lysis (Bajpai and Bajpai, 1992). Moreover, Yamada *et al.* (1987) and Bajpai *et al.* (1991) reported that *M. alpina* IS-4 and ATCC 32222 had their maximal production of lipids or PUFAs at 4 and 3 days incubation period, respectively.

Single cell oil production as affected by agitation speed: Obtained results (Table 5) indicate that the highest consumed sugar (68.8 and 73.3 g L^{-1}) was obtained at agitation speed of 175 and 200 rpm by *C. albicans* and *L. starkeyi*, respectively. Whereas, the lowest consumed sugar was found during the growth of the tested yeasts at 100 rpm. The agitation speed of 200 rpm showed

Table 5: Single cell oil and single cell protein production as affected by agitation speed

Yeast strain	Agitation speed (rpm)	Final pH	Consumed sugar (g L^{-1})	Cell weight (g L^{-1})	Oil weight (g L^{-1})	Growth yield efficiency (%)	Oil yield efficiency (%)	Oil (%)	dx/dt	dp/dt
Candida	100	4.2	54.8	5.1	0.8	9.3	1.46	15.68	0.1062	0.0167
albicans	125	4.2	57.3	5.6	1.2	9.8	2.09	21.43	0.1166	0.0250
NRRL Y-	150	4.6	62.7	5.9	1.6	9.4	2.55	27.12	0.1229	0.0333
12983	175	4.9	68.8	6.4	1.8	9.3	2.62	28.12	0.1333	0.0375
	200	5.0	68.0	6.5	1.8	9.6	2.65	27.69	0.1354	0.0375
Lipomyces	100	4.8	55.6	6.2	1.4	11.2	2.52	22.58	0.1291	0.0292
starkeyi	125	5.2	59.2	6.6	1.8	11.1	3.04	27.27	0.1375	0.0375
NRRL Y-	150	5.4	64.2	7.2	2.2	11.2	3.43	30.55	0.1500	0.0458
11557	175	5.4	68.9	7.8	2.6	11.3	3.77	33.33	0.1620	0.0542
	200	6.0	73.3	8.4	2.8	11.5	3.82	33.33	0.1750	0.0583

dx: Δ biomass, dt: Δ time, dp: Δ product

Table 6: Single cell oil and single cell protein production as affected by incubation temperature

Yeast strain	Incubation temperature °C	Final pH	Consumed sugar (g L^{-1})	Cell weight (g L^{-1})	Oil weight (g L^{-1})	Growth yield efficiency (%)	Oil yield efficiency (%)	Oil (%)	dx/dt	dp/dt
Candida	15	4.8	65.2	6.4	1.0	9.81	1.53	15.62	0.1333	0.0208
albicans	20	5.1	68.8	6.9	1.3	10.02	1.88	18.84	0.1437	0.0270
NRRL Y-	25	5.5	73.4	8.3	1.6	11.30	2.17	19.27	0.1729	0.0333
12983	30	5.2	65.3	7.2	0.8	11.02	1.22	11.11	0.1500	0.0167
Lipomyces	15	4.2	54.6	8.3	1.2	15.20	2.19	14.45	0.1729	0.0250
starkeyi	20	4.6	56.2	9.0	1.6	16.01	2.85	17.77	0.1870	0.0333
NRRL Y-	25	5.3	67.8	10.2	1.8	15.04	2.65	17.64	0.2125	0.0375
11557	30	5.0	60.4	9.6	1.8	15.89	2.98	18.75	0.2000	0.0375

dx: Δ biomass, dt: Δ time, dp: Δ product

the highest yield of both cell and oil weights by *C. albicans* and *L. starkeyi*. Likewise, the SCO productivities that being 0.037 and 0.058 g L^{-1} h^{-1} were found to be the best during the growth of *C. albicans* and *L. starkeyi* at 200 rpm, respectively. These results are comparable with the finding of Guilmanov *et al*. (2002) who found that the high agitation speed increases oxygen transfer rate during batch culture of *Candida bombicola*, then maximum values for both volumetric product formation (1 to 1.5 g L^{-1} h^{-1}) and sophorose yield (350 g L^{-1}) have been obtained. El-Fadaly *et al*. (2009) stated that, the agitation speed of 200 rpm was favorable for higher yield of SCO by *Cryptococcus curvatus* NRRL Y-1511. Previous study also showed that agitation speed has effect on the biomass and lipid content of oleaginous yeast, by which the higher concentration of dissolved oxygen connects with high agitation speed (Choi *et al*., 1982; Jacob, 1993).

Single cell oil production as affected by incubation temperature: The profile of final pH, consumed sugar, cell and oil weights as affected by incubation temperature has been determined (Table 6). Obtained data show that the highest final pH value (5.5) was obtained during the growth of *C. albicans* at 25°C, whereas the lowest pH value (4.2) was found during the growth of *L. starkeyi* at 15°C. The incubation temperature of 25°C was the optimum for the highest consumption of sugar (73.4 and 67.8 g L^{-1}) by *C. albicans* and *L. starkeyi*, respectively. The highest yield of cell weights being 8.3 and 10.2 g L^{-1} were obtained by *C. albicans* and *L. starkeyi* at 25°C, respectively. In addition, the highest SCO productivity that being 0.033 and

0.0.37 g L^{-1} h^{-1} was detected at 25°C by *C. albicans* and *L. starkeyi*, respectively. These data are coinciding with that obtained by Jang *et al.* (2005) who found that the growth yield of *M. alpina* increased at 15-25°C, but it decreased at temperature more than 28°C.

Rossi *et al.* (2009) found that the growth temperature did not influence the yield coefficients of both biomass and lipid production, but had significant effects on the growth rate and thus volumetric productivity. Kraisintu *et al.* (2010) also, found that the environmental conditions such as temperature has shown to be factors affecting lipid accumulation and composition. Further, Lindberg and Molin (1993) found that, 18°C was the optimum for high yield of poly unsaturated fatty acids by *M. alpina* CBS 343.66.

Screening of fatty acids composition by gas chromatography: The two tested yeast strains namely *C. albicans* NRRL Y- 12983 and *L. starkeyi* NRRL Y-11557 were allowed to grow at four individual different temperatures; 15, 20, 25 or 30°C, the obtained oils were then analyzed after methylation using GC instrument. The quantitative values (mg g^{-1}) of fatty acids, common name and symbol are illustrated in Table 7. About thirteen of saturated fatty acids (C6:0) (C8:0) (C10:0) (C11:0) (C12:0) (C13:0) (C14:0) (C15:0) (C16:0) (C17:0) (C18:0) (C20:0) (C22:0) were detected in

Table 7: Quantitative values of fatty acids content of single cell oil obtained by the two tested strains at different growth temperatures

			Fatty acid concentration expressed in mg g^{-1} at temperature of							
			C. albicans NRRL Y-12983				*L. starkeyi* NRRL Y-11557			
Common name of fatty acid	Symbol	Molecular formula	15°C	20°C	25°C	30°C	15°C	20°C	25°C	30°C
Caproic	C6:0	$C_6H_{12}O_2$	0.114	0.195	0.030	0.529	0.044	0.222	4.943	1.924
Caprylic	C8:0	$C_8H_{16}O_2$	0.277	0.554	0.024	1.094	0.146	0.441	0.714	2.225
Capric	C10:0	$C_{10}H_{20}O_2$	0.070	0.376	0.031	0.192	0.045	0.108	0.156	1.058
Undecanoic	C11:0	$C_{11}H_{22}O_2$	0.270	0.185	0.066	0.069	0.054	1.211	0.092	0.142
Lauric	C12:0	$C_{12}H_{24}O_2$	1.386	1.954	1.537	2.414	2.010	1.541	2.655	1.093
Tridecanoic	C13:0	$C_{13}H_{26}O_2$	1.476	0.127	0.102	0.063	0.008	0.000	0.128	0.165
Myristic	C14:0	$C_{14}H_{28}O_2$	3.542	3.384	6.434	6.057	6.500	2.372	5.676	1.926
Myristoleic	C14:1	$C_{14}H_{26}O_2$	0.280	0.256	0.603	0.411	0.445	0.270	0.320	0.203
Pentadecanioc	C15:0	$C_{15}H_{30}O_2$	0.696	0.244	0.772	4.634	0.579	0.143	0.394	0.198
Cis-10-pentadecanoic	C15:1	$C_{15}H_{28}O_2$	1.390	0.111	0.384	1.176	0.286	0.000	0.201	0.229
Palmitic	C16:0	$C_{16}H_{32}O_2$	13.33	16.47	20.54	15.31	23.98	18.20	21.14	11.53
Palmitoleic	C16:1	$C_{16}H_{30}O_2$	1.425	1.099	2.545	1.571	2.044	1.107	1.648	0.794
Heptadecanoic	C17:0	$C_{17}H_{32}O_2$	0.706	0.158	0.491	2.445	0.475	0.594	0.330	0.379
Cis-10-heptadecanoic	C17:1	$C_{17}H_{32}O_2$	1.234	0.122	0.271	1.227	0.241	0.341	0.174	0.575
Stearic	C18:0	$C_{18}H_{36}O_2$	3.983	4.951	6.660	6.374	8.328	4.634	6.133	2.144
Oleic	C18:1 ω9	$C_{18}H_{34}O_2$	22.08	27.60	54.26	50.62	37.40	26.92	33.68	14.03
Linoleic	C18:2 ω6	$C_{18}H_{30}O_2$	41.30	39.24	0.000	0.000	14.08	39.07	16.93	13.09
Linolenic	C18:3	$C_{18}H_{30}O_2$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Arachidic	C20:0	$C_{20}H_{40}O_2$	0.203	0.261	0.188	0.219	0.281	0.316	2.380	0.150
Cis-11-eicosenic	C20:1	$C_{20}H_{38}O_2$	0.650	0.000	0.275	0.000	0.181	0.948	0.174	0.542
Eicosadicnoic	C20:2 ω6	$C_{20}H_{36}O_2$	0.000	0.738	0.199	0.000	0.664	0.372	0.680	0.000
Arachidonic	C20:4	$C_{20}H_{32}O_2$	0.000	0.062	0.052	0.393	0.086	0.000	0.073	0.000
Erucic	C22:0	$C_{22}H_{42}O_2$	5.575	1.064	0.930	4.973	1.572	0.951	0.458	47.59
Decosahexaenoic	C22:6	$C_{22}H_{32}O_2$	0.000	0.833	3.898	0.219	0.535	0.223	0.723	0.000

the performed analysis as shown in Table 7. Four poly-unsaturated fatty acids; six of mono-unsaturated fatty acids (C14:1), (C15:1), (C16:1), (C17:1), (C18:1), (C20:1), two of di-unsaturated fatty acids (C18:2) and (C20:2) one of fourth unsaturated fatty acid (C20: 4) and one of sixth unsaturated (C22: 6) fatty acid were also detected. These results are comparable with profile of fatty acids of lipids produced by *Lipomyces starkeyi* (Angerbauer *et al*., 2008).

In case of yeast strain called *C. albicans* at 15°C, linoleic acid (C18: 2) was obtained at highest value to be 41.302 mg g^{-1} followed by oleic acid (C18: 1) of 22.083 then palmitic acid (C16: 0) recording 13.336 mg g^{-1} sample. The same trend was found with results obtained at 20°C but in different values being 39.249, 27.601 and 16.474 mg g^{-1} sample for linoleic acid (C18: 2), oleic acid (C18: 1) and palmitic acid (C16: 0), respectively. Oleic acid (C18 :1) and palmitic acid (C16 :0) showed to be the highest in fatty acids produced at 25°C and 30°C being 54.261, 20.544 and 50.625, 15.312 mg g^{-1} sample, respectively. Linolenic acid (C18: 3) was also not detected. Kraisintu *et al*. (2010) found that the major fatty acids of the cellular lipid of *Rhodosporidium toruloides* DMKU3-TK16 were oleic acid (41.54%), palmitic acid (22.49%), linoleic acid (15.12%) and stearic acid (14.56%). Tabulated results proved also that unsaturated fatty acids were superior to saturated ones taking into account the factor of incubation temperature.

Earlier study pointed out that the degree of unsaturation is higher in the microorganisms cultivated at lower temperature than in microorganisms cultivated at higher temperature (Hiruta *et al*., 1996). In addition; the differential in fat composition may be due to the genetic properties of the yeast strains, culture conditions and the kind of medium (Bednarski *et al*., 1986). The obtained unsaturated fatty acids values were 68.365, 70.071, 62.188 and 55.622% that corresponding to 31.635, 29.926, 37.811 and 44.375% of saturated ones. The values of fatty acids both saturated and unsaturated are plotted in Fig. 1. Furthermore, myristic acid (C14: 0), stearic acid (C18: 0) and erucic acid (C22: 0) were obtained in appreciated values at 15°C. Myristic acid (C14: 0) and stearic acid (C18: 0) were also obtained at 20°C. Myristic acid (C14: 0), palmitic acid (C16: 1), stearic acid (C18: 0) and decosahexaenoic acid (C22: 6) were also obtained at 25°C in appreciated values. Lauric acid (C12: 0), myristic acid (C14: 0), pentadecanoic acid (C15: 0), heptadecanoic acid (C17: 0), stearic acid (C18: 0) and erucic acid (C22: 0) were also obtained at 30°C in appreciated values.

For the other yeast strain *L. starkeyi*, at 15°C, oleic acid (C18: 1) was the highest value followed by palmitic acid (C16:0) being 37.408 and 23.988 mg g^{-1} sample, respectively. Linoleic acid (C18:2), oleic acid (C18:1) and palmitic acid (C16:0) were obtained in increasing order at 20°C being 39.073, 26.929 and 18.205 mg g^{-1} sample, respectively. The GC analysis of single cell oil obtained by *L. starkeyi*, at 25°C showed that oleic acid (C18:1) was superior followed by palmitic acid then

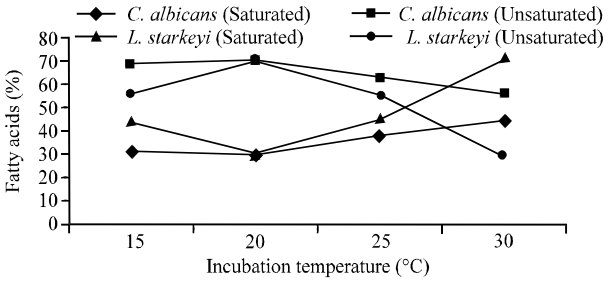

Fig. 1: Values of total saturated and unsaturated fatty acids of SCO obtained by *C. albicans* and *L. starkeyi* at different incubation temperatures

linoleic acid to be 33.687, 21.146 and 16.935 mg g^{-1} sample, respectively. Interestingly, this sample contained 4.943 mg g^{-1} of caproic acid (C6:0) and 2.380 mg g^{-1} of arachidonic acid beside 5.676 mg g^{-1} of myristic acid (C14:0). The fatty acid erucic (C22:0) was obtained at highest value at 30°C to be 47.597 mg g^{-1} sample followed by oleic acid (C18:1) of 14.031 mg g^{-1} and linoleic acid (C18:2) of 13.090 mg g^{-1}, while palmitic acid (C16:0) was found to be 11.536 mg g^{-1} sample.

Summarized data indicate that saturated fatty acids (C:0) represented about 44.029%, while the unsaturated fatty acids were 55.971% that obtained at 15°C. These data are coinciding with the finding of Jang *et al.* (2005) who found that low temperature stimulated the production of high unsaturated fatty acids, such as arachidonic acid, eicosapentaenoic acid, linoleic acid and γ-linolenic acid. Moreover, previous studies showed that many species of the genus *Mortierella* have been found to yield high amount of polyunsaturated fatty acids depending upon the fermentation media and culture conditions (Jang *et al.*, 2000; Yu *et al.*, 2003).

The values of 69.263, 54.797 and 29.463% represented the total unsaturated fatty acids that corresponding to 30.739, 45.203 and 70.537% of the total saturated fatty acids obtained at 20, 25 and 30°C, respectively. Both tridecanoic and cis-10-pentadecanoic acids were absent at 20°C. Unfortunately, the polyunsaturated fatty acid, linolenic acid (C18.3) was absent in this sample. Furthermore, arachidonic acid (C20:4) was absent at 20 and 30°C. Additionally, both eicosadiecnoic acid and decosahexaenoic acid were also absent at 30°C. The total values of saturated and unsaturated fatty acids are plotted in Fig. 1.

CONCLUSION

It can be concluded that the single cell oils obtained in this investigation by the oleaginous yeasts could be used as alternative bio-procedure for SCO, since this study showed the efficiency of *C. albicans* NRRL Y-12983 and *L. starkeyi* NRRL Y-11557 in assimilation of some agro-industrial by-products and production of SCO. Data also showed that sugar beet molasses and glucose syrup were the best for SCO production by the tested yeast. In addition, the initial pH (6), incubation temperature (25°C), agitation speed (200 rpm) and incubation period (72 h) were the optimum values for highest SCO productivity. Obtained results also showed that palmitic, oleic and linoleic acid are the major fatty acids that occurred in highest amount of SCO produced by the two yeast strains.

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