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THE POTENTIALITY OF SACCHAROMYCES CEREVISIAE TO PRODUCE SINGLE CELL OIL (SCO) FROM WHEAT STRAW AND MOLASSES

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ABSTRACT

The study aimed to explore the use of isolated *Sacchromyces cerevisiae* for single cell edible oil production using wheat straw and molasses as a cheaper source of nitrogen and sugars. A total of 10 samples of Sacchromyces cerevisiae were used, isolated from rotten fruit and fruit juice, the effectiveness of them to produce the single cell oil was studied, when the yeast was grown on wheat straw, detoxified liquid hydrolysate (DLH) and nondetoxified liquid hydrolysate (NDLH) and molasses. The productivity of single cell oil produced by Sacchromyces cerevisiae strains was different according to the sources of isolation. Sacchromyces cerevisiae isolated from rotten apple gave high oil productivity 84.05%, of lipids on sugar cane molasses medium and 50.7 % on wheat straw medium.

Keywords: Oleaginous yeast, single cell oil, DLH: detoxified liquid hydrolysate.

INTRODUCTION:

Microbial oils, namely, single cell oils (SCO), which are lipids that are produced by oleaginous microorganisms, have been of potential interest to many researchers in the past decades due to their significant functions and specific characteristics [1]. Traditionally, microorganisms, which include bacteria, yeasts, molds and microalgae that can accumulate lipids to more than 20% of their dry weight are considered oleaginous microorganisms [2]. In the subsequent 20 years, such biochemical processes and SCO production were of interest to more people because SCO could play critical roles in maintaining human health [3] by replacing some expensive materials such as cocoa butter [4]. During those years, the process of lipid accumulation was more completely elucidated, and the studies varied. Furthermore, researchers continued to pay attention to biochemical mechanisms to explain how microorganisms accumulate lipids in their bodies [2, 5 and 6]. It is obvious that single cell oil (SCO) oil will play a more critical role in the future, and low-cost substrates for SCO production will play a key role in the industrialization of SCO production. Several scientists have reviewed SCO and its production over the past decades [7]. Recently, there are alternative resources of edible oil, the single cell oil (microbial oil) from low cost substrates of which composition is similar to traditional vegetable oils [8]. Cost of production for oil seeds and oils processing increased gradually due to adoption of economic policies (Liberalization policies removal of subsidies of agricultural Inputs), cost of production and processing highly increased due to high prices of agricultural inputs especially the tradable ones [9]. Because the prices of sesame, olive, sunflower and ground nut oils are on the rise, a cheaper alternative may be production of edible oil using the residues of some crops e.g. wheat, potato and sugarcane. Therefore, the production of single cell oil as a cheaper alternative is one of the most promising studies in the field of food and therapeutic oils. Under the premise of increasing edible oil prices, the single cell oil producing by oleaginous yeast grown on cheaper substrate (by-product) is considered as a promising replacement for edible oil. Advantages of microbial oil production compared to plant oil is the short life cycle of microbes and the possibility of a production process not influenced by external factors such as venue, season or climate [10]. Furthermore, less land is needed for microbial production than for conventional agricultural production [11]. The studies in this field involved the key enzymes of those processes and their regulation of lipid

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accumulation [12 and 13], and key intermediates for lipid biosynthesis [14]. Additionally, screening for optimal oleaginous microorganisms became a key mission of many scientists in the field of SCO production [15]. Other related and interesting aspects such as detective methods for SCO were also explored [16] Grasses, trimmings of lawns, other agriculture wastes, industrial, domestic, food and urban solid wastes are produced at a rate of 43 million tons/year. Utilization by recycling of these wastes would not only aid in pollution abatement but can also serve as a vital source of energy and food for the future [17]. These waste products containing lignocelluloses' biomass are the most abundant organic raw material and are being used widely in fermentation industry as a microbial substrate for the production of many value added products including hydrolytic enzymes [18]. Cost substrate for SCO production will play a key role in the industrialization of SCO production. Many efforts focused on using low-cost materials as media for SCO production. Generally, two types of lipid synthesis exist in oleaginous microorganisms: the "de novo" and "ex novo" lipid accumulation processes [19]. The former process is usually carried out on hydrophilic materials and usually requires nitrogen-limited culture conditions. In contrast, "ex novo" lipid production is the production of SCO through fermentation on hydrophobic materials. Based on this point, the low-cost substrates that are used for microbial oil production can be divided into hydrophilic and hydrophobic [20]. Wheat straw (WS) is a lignocellulosic material that is an abundant by-product in many wheat production regions. In 2008 the worldwide wheat production was estimated to be over 650 million tonnes, thus about 850 million tons of wheat straw were produced annually based on the straw/crop ratio of 1-3 [21]. In fermentation industry, WS can be used as a substrate for the production of vast range of hydrolytic enzymes, medicines, biofuel and other metabolites. It is the cheapest or low cost source of natural substrate [22]. WS consists of 35-45% cellulose, 20-30% hemicellulose, and 8-15% lignin [23].

Cellulose consists of glucose while hemicellulose contains predominant amounts of pentoses and a few hexoses. Although these two carbohydrate components in the biomass can be converted to fermentable sugar monomers for biofuele and microbial oil production, the direct enzymatic hydrolysis is impeded due to the physico-chemical and structural cell wall composition of the biomass. Thus, biomass pretreatment prior to enzymatic hydrolysis is essential to enhance the accessibility of cellulase to cellulose. Among various chemical pretreatment methods, dilute sulfuric acid is the most commonly applied catalyst [24]. Although, there are several studies on converting hemicelluloses hydrolysate into lipids by oleaginous yeast strains [25], these strains were unable to efficiently produce lipids in the presence of the inhibitors in the hydrolysate. So a detoxification treatment was required prior to the fermentation [7]. Sugarcane and molasses have been used for SCO production [26]. More recently, *Cunninghamella echinulata* showed great potential in the decolorization-detoxification of waste molasses and in efficiently using molasses for SCO production [27]. Oleaginous microorganisms can grow well on molasses medium due to its high sugar content [26]. Therefore, the molasses medium and wheat straw medium were used to be more effective for promotion of the accumulation of substantial amount of lipids by *Sacchromyces cerevisiae*.

MATERIALS AND METHODS:

Samples:

Wheat straw was collected from cultivated wheat fields after harvesting. The straw was washed, air dried, and milled to pass through a 2 mm sieve, the processed straw was then sealed in plastic bags and stored at room temperature. Sugarcane molasses was obtained from the Kenana sugar factory and was diluted to the acceptable sugar level (12%) for the growth of yeast and then was sealed in bottles and stored at room temperature, then closed and immediately transported to laboratory.

Single cell oil production in wheat straw substrate:

The single cell oil was produced according to the method of Chen *et al*., 2009, In order to investigate the capability of *Sacchromyces cerevisiae* to produce lipids using wheat straw as a growth substrate, ten samples of *S. cerevisiae* were isolated for different sources, and used as microbial lipid producers. Wheat straw was pretreated with dilute sulfuric acid (2%) and the liquid fraction obtained was separated via vacuum filtration and divided into two portions. One fraction was detoxified and the other non-detoxified liquid hydrolysate as described by [28], these were then used as substrates in yeast fermentation. And finally the lipids produced were quantified.

Preparation of the dilute acid pretreated wheat straw hydrolysate:

The diluted acid pretreatment condition used was similar to those described by [25 and 29]. Wheat straw was suspended and stirred at room temperature in 2% (v/v) dilute sulfuric acid solution at a solid loading of 10 % (w/v). The mixture was then treated in an autoclave at 121°C for 60 min. After cooling, the autoclaved liquid hydrolysate was separated by centrifugation and vacuum filtration and then stored at 4°C prior to use.

Preparation of the detoxified liquid hydrolysate (DLH):

The original hydrolysate was first heated to 42°C while stirring using a stir bar. Calcium hydroxide was then added to increase the pH to 10.0 in a process called overliming. The temperature of the hydrolysate increased to 50-52°C by addition of calcium hydroxide, and thereafter the mixture was maintained at 50°C and stirred for 30 min using the heater stir plate, followed by filtration using a 0.22 membrane (Millipore, MA), and the filtrate was allowed to cool to 30°C, then re-acidified to pH 5.5 with sulfuric acid (2%), followed by 0.22 filtration to remove any precipitate formed. This detoxified liquid hydrolysate was then ready for use as a fermentation substrate.

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Preparation of the non-detoxified liquid hydrolysate (NDLH):
Calcium hydroxide was added to the original liquid hydrolysate at room temperature until the pH was 5.5. Then the mixture was filtered using a 0.22 membrane. This filtrate was prepared as the non-detoxified liquid hydrolysate and ready for use as a fermentation substrate.

Yeast strains cultivation in NDLH and DLH:
Single cell oil producer (yeasts) were grown in the medium containing 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, and 10 g/L xylose. The medium was first incubated at 30ºC for 24 h as a preculture. Inoculums (10%, v/v) were then added to the culture medium, which included 50 ml each of either non-detoxified liquid hydrolysate (NDLH) or detoxified liquid hydrolysate (DLH), as well as 0.4 g/L $MgSO_4$,$7H_2O$, 2 g/L KH_2PO_4, 0.003 g/L $MnSO_4.H_2O$, 0.0001 g/L $CuSO_4.5H_2O$, and 1.5 g/L yeast extract.

Single cell oil production in sugarcane molasses substrate:
Singe cell oil in molasse was carried out according to the method of [25 and 29], using the dilute acid pre-treated sugarcane molasses hydrolysate. Sugarcane molasses was suspended and stirred at room temperature in 2% (v/v) dilute sulfuric acid solution at a solid loading of 10% (w/v). The mixture was then treated in an autoclave at 121°C for 60 min. After cooling, the autoclaved liquid hydrolysate was separated by centrifugation and vacuum filtration and then stored at 4°C prior to use.

Preparation of the sugarcane molasses detoxified liquid hydrolysate (SDLH):
The sugarcane molasses detoxified liquid hydrolysate (SDLH) was prepared as mentioned above.

Yeasts Strains cultivation in SDLH:
Yeast was cultivated as mentioned above.

Dry cell weight determination:
The biomass was determined by the method described by [30], 5 ml cell suspension sample was centrifuged at 2500 rpm for 5 min. The cell pellet was then washed twice with distilled water, dried in a pre-weighed aluminium dish at 105°C for 3 h, and the final mass was expressed as dry cell weight (DCW).

Oil extraction:
Yeast cells were harvested from fermentation broth by centrifugation at 8000 rpm for 10 min and freezed overnight, and then were transferred to 50 ml of hexane; oil was extracted by shaking in 28°C for 48 h using a stir bar in separator according to the procedure of [31].

RESULTS AND DISCUSSIONS:

Oil production in wheat straw substrate:
Table 1 and Fig 1 show the biomass of *Sacchromyces strains* on DLH and NDLH wheat straw. *Sacchromyces strain* isolated from rotten apple (A_1) was found to give the highest biomass of mean value (1.905 g/l) in NDLH, while the isolates from air (Ai_1) gave the lowest biomass mean value (1.190 g/l) in DLH. The results showed high biomass performance when using non detoxified liquid hydrolysate in all samples, with the exception of strains which were isolated from banana juice (Bj_1, Bj_2) and orange juice (Oj_2). Results indicated that the non-detoxified hydrolysate did not have a negative impact on biomass. Variation in biomass value depends on the *Sacchromyces strains* used under this investigation. The data showed significant difference (P≤0.05) among samples. These results were not in agreement with the findings of [32], who reported biomass decreased in *Rhodosporidium toruloides*, when grown on DLH wheat straw and [33], who used five oleaginous yeast strains; *Cryptococcus curvatus*, *Rhodotorula glutinis, Rhodosporidium toruloides, Lipomyces starkeyi* and *Yarrowia lipolytica*, which were evaluated by using hydrolysate pre-treatment of wheat straw as substrates. Their results showed that the growth of all of the selective yeast strains, *C. curvatus* showed the highest lipid concentrations in medium on both the detoxified (4.2 g/L) and non-detoxified (5.8 g/L) hydrolysates, the highest biomasses obtained were 17.2 g/L in NDLH and 15.6 g/L in DLH, achieved by *C. curvatus*. However, their results were very high compared to the result of this study.

Table 1: Biomass production (g/l) of *Sacchromyces cerevisiae* on detoxified and non-detoxified liquid hydrolysate wheat straw

S. Cerevisiae Samples	DLH	NDLH
M_1	1.810^{abcd}±0.13	1.895^{ab}±0.02
M_3	1.790^{cd}±0.00	1.900^{a}±0.00
Gf_2	1.510^{e}±0.03	1.795^{bcd}±0.04
A_1	1.295^{g}±0.01	1.905^{a}±0.01
A_3	1.260^{g}±0.01	1.850^{abc}±0.03
B_3	1.240^{g}±0.00	1.805^{abcd}±0.01
Ai_1	1.190^{g}±0.06	1.225^{g}±0.06
Bj_1	1.270^{g}±0.03	1.195^{g}±0.02
Bj_2	1.295^{g}±0.01	1.195^{g}±0.06
Oj_2	1.470^{ef}±0.07	1.450^{ef}±0.02
P-value	0.00^{**}	
$Lsd_{0.05}$	0.09193	
SE±	0.03162	

Values are mean ±SD; Values bearing different superscripts in columns and rows are significantly different (P≤0.05) according to DMRT. DLH and NDLH are detoxified and non-detoxified liquid hydrolysate. M_1 to oj_2 are the yeast samples isolated from rotten fruits (mango, grapefruit, apple and banana), air, banana juice and orange juice, respectively.

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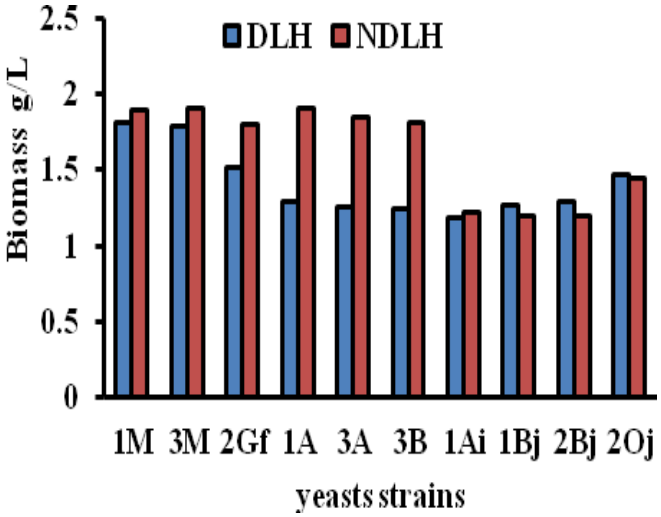

Fig. 1: Biomass production of yeast produced on detoxified and non-detoxified liquid hydrolysate wheat straw [DLH and NDLH are detoxified and non-detoxified liquid hydrolysate. M_1 to oj_2 are the yeast samples isolated from rotten fruits (mango, grapefruit, apple and banana), air, banana juice and orange juice, respectively].

Table 2 and Fig 2 show the oil accumulation of *S. cerevisiae*. The highest mean value was found in sample A_1 which was isolated from rotten apple (0.6567g) in DLH, while the lowest one was reported in sample Bj_2 which was isolated from banana juice (0.1707g) in NDLH (Table 2). The results showed that the oil accumulation value was significantly (P≤0.01) higher when the *S. cerevisiae* samples were grown on DLH than NDLH, this is because in dilute sulfuric acid pre-treated wheat straw hydrolysate various degradation products were present, which mainly included acetic acid from acetyl groups in hemicellulose, furfural from pentose, and 5- hydroxy methyl furfural (HMF) from hexose, and these compounds strongly inhibit microorganisms during the fermentation process, so a detoxification treatment was required prior to the fermentation [25]. Oil production (%) of *S. cerevisiae* samples are shows in Table 3 and Fig 3. The highest mean level (50.7 %)was found in sample A_1, followed by sample A_3 (48.5 %) in DLH which was isolated from rotten apple, while the lowest mean value (14.5%) was found in sample Gf_2 in NDLH which was isolated from grapefruit. Variation in oil production value depends on the *S. cerevisiae* isolates and DLH, NDLH liquid hydrolysate wheat straw used. The data showed significant difference (P≤0.05) when the single cell oil producer was grown on DLH and NDLH. The oil production was higher when using detoxified liquid hydrolysate as substrate for producing single cell oil than using non- detoxified liquid hydrolysate. The high percentage of oil produced in DLH oil sample indicates the importance of detoxification process, this because the oleaginous strains were unable to efficiently produce lipids in the presence of the inhibitors in the hydrolysate. A detoxification treatment was, therefore, required prior to the fermentation [25]. These results were not in agreement with the findings of [33], which used this hydrolysate pre-treatment of wheat straw as substrates. Their results showed the lipid contents of 33.5% and 27.1% in the NDLH and DLH, respectively. Lipid concentrations in all *S. cerevisiae* strains were slightly higher in the NDLH than in the DLH, indicating that the non-detoxified hydrolysate did not have a negative impact on lipid accumulation.

Table 2: Oil content (g) when using detoxified and non-detoxified liquid hydrolysate wheat straw as produced by *Sacchromyces cerevisiae*from different sources

S. Cerevisiae Samples	DLH	NDLH
M_1	0.3550^{fghi}±0.04	0.3178^{hij}±0.01
M_3	0.3647^{fgh}±0.03	0.3314^{hi}±0.01
Gf_2	0.3063^{hij}±0.03	0.2533^{ijk}±0.06
A_1	0.6567^{a}±0.03	0.3484^{ghi}±0.00
A_3	0.6116^{abc}±0.01	0.2827^{hij}±0.00
B_3	0.5492^{cd}±0.00	0.4412^{rfg}±0.00
Ai_1	0.4334^{efg}±0.01	0.2970^{hij}±0.00
Bj_1	0.4553^{def}±0.00	0.1749^{k}±0.00
Bj_2	0.3217^{hij}±0.21	0.1707^{k}±0.00
Oj_2	0.6278^{ab}±0.05	0.5158^{cde}±0.01
P-value	0.00^{**}	
$Lsd_{0.05}$	0.09193	
SE±	0.03162	

Values are means±SD; Values bearing different superscripts in columns and rows are significantly different (P≤0.05) according to DMRT. DLH and NDLH are detoxified and non-detoxified liquid hydrolysate. M_1 to Oj_2 are the yeast samples isolated from rotten fruits (mango, grapefruit, apple and banana), air, banana juice and orange juice, respectively.

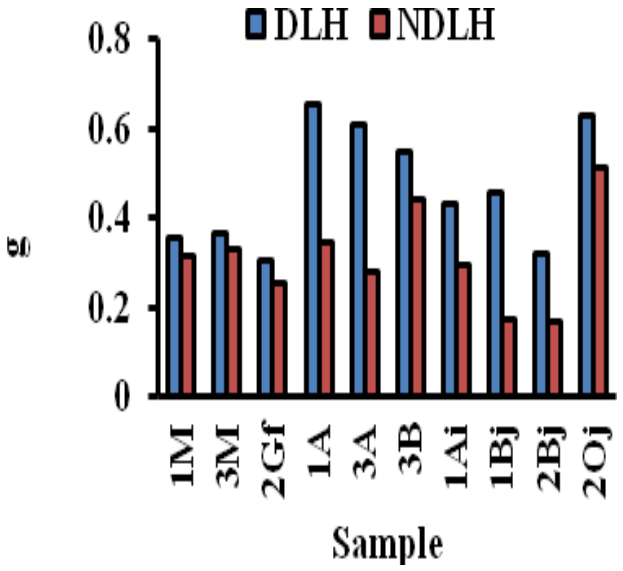

Fig. 2: Oil content of using detoxified and non-detoxified liquid hydrolysate wheat straw as produced by oleaginous yeast from different sources. [DLH and NDLH are detoxified and non-detoxified liquid hydrolysate. M_1 to oj_2 are the yeast samples isolated from rotten fruits (mango, grapefruit, apple and banana), air, banana juice and orange juice, respectively].

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Table 3: Oil production (%) of *Sacchromyces cerevisiae* produced on using detoxified and non-detoxified liquid hydrolysate on wheat straw

S. Cerevisiae Sample	DLH	NDLH
M_1	$22.20^{fg}\pm0.00$	$16.75^{jkl}\pm0.64$
M_3	$20.30^{gh}\pm1.41$	$17.35^{ijk}\pm0.35$
Gf_2	$20.05^{ghi}\pm1.34$	$14.05^{lm}\pm2.90$
A_1	$50.65^{a}\pm1.77$	$18.55^{hij}\pm0.35$
A_3	$48.50^{ab}\pm0.28$	$15.25^{klm}\pm0.35$
B_3	$44.25^{cd}\pm0.21$	$24.40^{f}\pm0.00$
Ai_1	$36.40^{e}\pm1.13$	$24.80^{f}\pm0.42$
Bj_1	$35.80^{e}\pm0.99$	$14.60^{lm}\pm0.14$
Bj_2	$36.50^{e}\pm0.00$	$14.45^{lm}\pm0.07$
Oj_2	$42.60^{d}\pm1.27$	$35.45^{e}\pm0.78$
P-value	0.00^{**}	
$Lsd_{0.05}$	2.518	
SE±	0.8663	

Values are means±SD; Values bearing different superscripts in columns and rows are significantly different (P≤0.05) according to DMRT. DLH and NDLH are detoxified and non-detoxified liquid hydrolysate. M_1 to oj_2 are the yeast samples isolated from rotten fruits (mango, grapefruit, apple and banana), air, banana juice and orange juice, respectively.

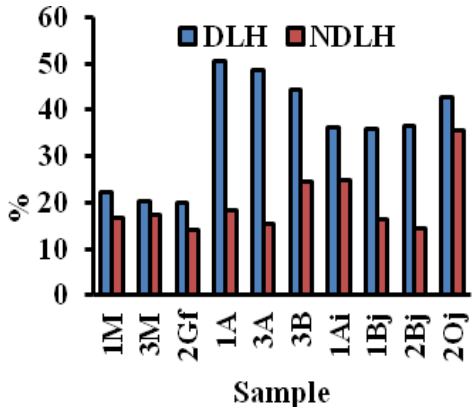

Fig. 3: Oil production of yeast produced on using detoxified and non-detoxified liquid hydrolysate on wheat straw [DLH and NDLH are detoxified and non-detoxified liquid hydrolysate. M_1 to oj_2 are the yeast samples isolated from rotten fruits (mango, grapefruit, apple and banana), air, banana juice and orange juice, respectively].

Oil production using sugar cane molasses substrate:

The highest mean value of biomass (2.57 g/l) was detected in the sample isolated from rotten banana (Bj_2), while the lowest mean value (1.50g/l) was detected in sample **A_3** which isolated from rotten apple (Table 4 and Fig 4).

No significant differences in (P≤0.05) in biomass were observed among *S. cerevisiae* starins in this study. The biomass ranges from 1.50 to 2.57 (g/l) was lower than the findings (8.27g/L) of [34], who used mixed cultures of the oleaginous yeast for microbial oil production using sugar cane molasses as carbon substrate. The highest oil value was found in sample A_1 which was isolated from rotten apple (1.523g/l), while the lowest one was found in sample M_3 which isolated from rotten mango (0.980g). The oil range from 0.980 to 1.523 was higher than the findings (0.920g) of {34}, Yeast sample which was isolated from rotten apple (A_3) gave the highest oil production mean value (84.05%), followed by sample (A_1), isolate from rotten apple produced 77.75% oil content. The lowest one was found in sample Bj_2 isolated from banana juice (41. 65%).The data showed significant difference (P≤0.05) among *Sacchromyces cerevisiae* isolates. These results were found to be higher than the findings of [35], who found that the *Saccharomyces cerevisiae* strains accumulated more than 40 % of lipids on sugar cane molasses.

Table 4: Production of oil using sugarcane molasses substrate

S. Cerevisiae Samples	Biomass (g/l)	Oil (g/l)	Oil production (%)
M_1	$1.700^{a}\pm0.06$	$1.138^{ab}\pm0.25$	$67.00^{abc}\pm16.69$
M_3	$2.151^{a}\pm0.20$	$0.980^{b}\pm0.06$	$46.35^{bc}\pm6.15$
Gf_2	$1.612^{a}\pm0.14$	$1.113^{ab}\pm0.09$	$68.85^{abc}\pm0.92$
A_1	$1.987^{a}\pm0.80$	$1.523^{a}\pm0.51$	$77.75^{ab}\pm5.59$
A_3	$1.500^{a}\pm0.14$	$1.253^{ab}\pm0.03$	$84.05^{a}\pm5.73$
B_3	$2.605^{a}\pm0.56$	$1.125^{ab}\pm0.12$	$44.70^{bc}\pm14.28$
Ai_1	$1.730^{a}\pm0.35$	$0.9835^{b}\pm0.00$	$58.10^{abc}\pm12.16$
Bj_1	$2.545^{a}\pm0.01$	$1.107^{ab}\pm0.19$	$43.55^{bc}\pm7.57$
Bj_2	$2.570^{a}\pm0.48$	$1.040^{b}\pm0.13$	$41.65^{c}\pm12.66$
Oj_2	$2.340^{a}\pm0.68$	$1.030^{b}\pm0.11$	$46.60^{bc}\pm18.24$
P-value	0.4854^{NS}	0.0359^{*}	0.0141^{*}
$Lsd_{0.05}$	1.173	0.4042	30.44
SE±	0.3841	0.1323	9.962

Values are means±SD; Values bearing different superscripts in columns and rows are significantly different (P≤0.05) according to DMRT. DLH and NDLH are detoxified and non-detoxified liquid hydrolysate. M_1 to oj_2 are the yeast samples isolated from rotten fruits (mango, grapefruit, apple and banana), air, banana juice and orange juice, respectively.

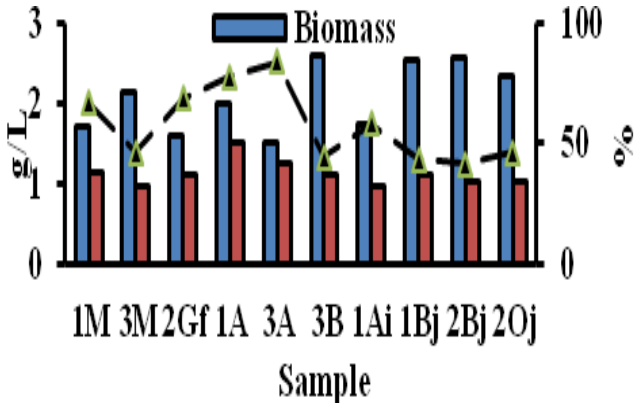

Fig. 4: Production of oil using sugarcane molasses substrate [DLH and NDLH are detoxified and non-detoxified liquid hydrolysate. M_1 to oj_2 are the yeast samples isolated from rotten fruits (mango, grapefruit, apple and banana), air, banana juice and orange juice, respectively].

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CONCLUSION

It could be concluded form this study, that the productivity of single cell oil produced by *Sacchromyces cerevisiae* was different according to the sources of isolation. The highest productivity of oil (50%) was achieved by *Sacchromyces cerevisiae,* when using wheat straw as substrate, while the oil production was 84 % when using molasses as substrate. Molasses medium was found to be more effective for promotion of the accumulation of substantial amount of lipids by *Sacchromyces cerevisiae*.

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