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EFFECT OF SOME SUGARS AND AGRO-INDUSTRIAL BY-PRODUCTS ON CAROTENOIDS PRODUCTION BY SOME YEAST STRAINS OF *RHODOTORULA SPP.*

Selim,A. E. I.; M. M. Kassem; Samia M. M. Bayoumy; M. M. A. El-Sawah and Dina F. I. Ali

Dept. of Agric. Microbiol., Fac. of Agric., Mansoura Univ., Mansoura , Egypt.

ABSTRACT

The production of carotenoids by *Rhodotorula glutinis* NRRL Y-842, *R. armeniaca* NRRL Y-17201, *R. mucilaginosa* NRRL Y-844, *R. bogoriensis* NRRL Y-5980 or *R. aurantiaca* NRRL Y-1584 on glucose as a carbon source were investigated at different incubation periods at 30°C in shaking incubator. Among the different yeast strains, *R.glutinis* NRRL Y-842 proved to be the most active producer during the fermentation period, the maximum values of carotenoids produced by *Rhodotorula* strains were 2328.29µg/L at 96hr., 10.99 µg/L at 96hr., 10.99 µg/L at 96hr., 71.13 µg/L at 120hr. and 62.73 µg/L at 96hr., respectively. Using sucrose, lactose, maltose or fructose as a carbon source, *R. glutinis* NRRL Y-842 produced 732.45µg/L at 72hr., 488.88µg/L at 72hr., 1704.97µg/L at 96hr. and 2166.01µg/L at 96hr., respectively. When agro- industrial by-products, namely, untreated sugarcane molasses, untreated beet molasses, treated sugarcane molasses, treated beet molasses, untreated vinasse, treated vinasse, glucose syrup, soy bean flour extract (SFE) and maize flour extract (MFE) were also used as a carbon source, the maximum values of carotenoids produced by *R. glutinis* NRRL Y-842 were 1772.19 µg/L at 120 hr., 1571.40µg/L at 144 hr., 1002.01µg/L at 120 hr., 679.00µg/L at 96 hr., 916.65µg/L at 120 hr., 452.89µg/L at 120 hr., 1831.36µg/L at 96 hr., 426.80µg/L at 48 hr. and 340.79µg/L at 48 hr., respectively.

Keywords: Carotenoids, *Rhodotorula spp.*, Molasses, vinasse.

INTRODUCTION

Carotenoids are a group of natural pigments produced by a wide range of microorganisms and plants. They are used commercially as food colorants and for their pharmaceutical and chemical role, not only they act as vitamin A precursors, but also for their coloring, antioxidant, possible tumor- inhibiting activity, involvement in the visual attraction of animals such as flower pollinators or mating partners(Johnson and Schroeder,1995;Ferrao and Garg,2011). Many carotenoids are being produced by chemical synthesis, which yields products that are pure and cheap (Gordon and Pauerfeind, 1982).

The international market of carotenoids has been met mainly by synthetic carotenoids with structures identical to those of natural carotenoids, but there is growing demand for natural sources. Microbial carotenoids have been studied and their potentials recognized over the years. Several microorganisms, including bacteria, algae, moulds and yeasts of the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces* and *Phaffia* are able to produce carotenoids naturally(Frengova and Beshkova, 2009). The

synthesis of different natural important carotenoids by several yeasts species belonging to the genera *Rhodotorula and Phaffia*, has led to consider these microorganisms as a potential pigment sources (Catalina Voaides and Dima, 2012).

The commercial utilization of microorganisms with bio-technological potential to produce carotenoids is presently limited by the high cost production. However, the cost of carotenoid production can be minimized by the use of inexpensive industrial by-products as nutrient sources (Aksu and Eren, 2005).

The aim of this study was to examine the potential of some sugars and some agro-industrial by-products as substrates for the production of carotenoids by some yeast strains belonging to the genus *Rhodotorula*.

MATERIALS AND METHODS

I. Yeast strains

Five yeast strains, namely, *Rhodotorula glutinis* NRRL Y-842, *Rhodotorula armeniaca* NRRL Y-17201, *Rhodotorula mucilaginosa* NRRL Y-844, *Rhodotorula bogoriensis* NRRL Y-5980 and *Rhodotorula aurantiaca* NRRL Y-1584 were kindly obtained from Cairo Mircen, CAIM, Faculty of Agriculture, Ain- Shams Univ., Cairo, Egypt. These strains were subcultured on YM (ATCC medium 200) slants (dextrose 10.0 g, peptone 5.0 g, yeast extract 3.0 g, malt extract 3.0g, agar 15.0 g, distilled water 1.0 L) at 28° C, maintained at 4° C and subcultured every 3 weeks.

II. Production medium

25.0 g glucose, 10.0 g yeast extract, 2.0 g K_2HPO_4, 2.0 g KH_2PO_4, 0.1 g $MgSO_4$.7H2O, 1.0 L distilled water. The pH of the medium was adjusted at 6 (Bhosale and Gadre, 2001a). The medium was sterilized at 100 °C for 20 min. 3 times with 24hr. interval. Furthermore 400 µg/L of thiamine and 23 µg/L of P-aminobenzoic acid were added to the production medium in case of all strains except *Rhodotorula glutinis* NRRL Y-842 (Peterson *et al.*, 1958).

III. Preparation of standard inoculum

Inocula were obtained from cultures grown on YM slants at 28°C for 48 hr. A loop of the yeast cells was transferred to 5ml of production medium and incubated at 28°C for 24 hr., transferred to 100ml of fermentation medium in 250ml Erlenmeyer flask. Inoculated flasks were incubated in a rotary shaking incubator (Lab-Line Incubator-Shaker) at 30°C/150 rpm. Samples were taken every 24 hr. interval through 6 days, and the following parameters of fermentation were studied.

III.1. Time course

To investigate carotenoids production by *Rhodotorula* strains, the cultures of the used five strains cultivated on fermentation medium were incubated for 6 days at 30°C. The cell dry weight, carotenoids, pH and consumed sugars were investigated daily for each strain.

III.2. Carbon source

III.2.1. Sugars

To investigate the effect of some sugars on carotenoids production by *Rhodotorula glutinis* NRRL Y-842, glucose was replaced by sucrose, lactose, maltose or fructose. Each source was added to the fermentation medium equivalent to 25 gm glucose /L. The final volume of each fermentation medium was adjusted at pH 6, distributed in flasks, sterilized at 100°C for 20 min. 3 times with 24hr. interval, inoculated and incubated at 30°C for 6 days.

III.2.2. Molasses

According to the method of Bhosale and Gadre (2001b), sugarcane and beet molasses were used for fermentation experiments. They were diluted 2 folds (W/V) using distilled water. Then each was divided into 2 parts, the pH of the first part was adjusted to 2.0 with 5N HCl and it was held in boiling water bath for 40 min. for sucrose hydrolysis. After hydrolysis, the solution was cooled to room temperature and its pH was adjusted to 6.0 with 1N NaOH, The precipitate was removed by centrifugation. Total reducing sugar (TRS) was assayed spectrophoto-metrically using Somogyi (1952) method in both treated and untreated molasses. After addition of 2 g/L of both K_2HPO_4 and KH_2PO_4, the solutions were sterilized at 100°C for 20 min. 3 times with 24hr. interval and used as a fermentation medium.

III.2.3. Glucose syrup

When glucose syrup was used for fermentation experiments, it was diluted 2 folds (W/V) using distilled water, TRS were measured, and then they were added to the fermentation medium instead of glucose in equivalent concentration. The medium was sterilized at 100°C for 20 min. 3 times with 24hr. interval.

III.2.4. Soy bean flour and maize flour

According to Buzzini and Martini(1999), 1.5 L. Sulfuric acid (0.2 N) was added to 250 g of each of soy bean flour and maize flour, then autoclaved on 121° C for 15 min., cooled and centrifuged at 5000 rpm for 10 minutes . In case of MFE, any carotenoid traces were extracted using di-ethyl ether in order to remove possible traces of carotenoids, then centrifuged and added to the fermentation medium without glucose to reach a TC concentration of 25g/L. The medium was sterilized at 100°C for 20 min. 3 times with 24hr. interval.

IV. Extraction of carotenoids

According to the method of **Park *et al.* (2005)**, modified by **Ali (2013)**, to extract the carotenoids, 50ml of each fermented liquor was centrifuged at 3500 rpm for 15 min., after decanting the supernatant, the precipitate was washed twice by distilled water and the washing-water was discarded after centrifugation every time. The precipitated cells were broken up with a glass rod after adding 10 ml of 0.5N HCl, boiled in water bath for 15 min., cooled in ice water for 10 minutes. For extraction of the pigments, measured amounts of acetone were added and the cells stirred vigorously. To ensure complete extraction of the pigments, more amounts of acetone were added until cells

turn colorless. Amounts of di-ethyl ether equal to those of acetone were added to the previous, and moved to a separation funnel. Then amounts of cold NaCl solution (15%) were added for purification of di-ethyl ether layer, and then the di-ethyl ether layer was taken and assayed spectrophotometrically at 455 nm. (JENWAY 6305 UV/vis. Spectrophotometer). The standard curve was blotted using β-carotene as a standard substrate. Amounts of carotenoids were calculated using standard curve.

V. Fermentation aspects

At certain time intervals (24, 48, 72, 96, 120 and 144 hr.), the following fermentation aspects were done.

V.1. Cell dry weight

The cell dry weight was determined in 50 ml of the fermented culture by centrifugation at 3500 rpm for 15 min., washing the sediment twice with distilled water and dried at 85 °C to constant weight.

V.2. Residual sugars

Residual sugars were determined in the centrifuged fermented liquor after removing cells, according to the method of Somogyi (1952) using glucose as a standard.

V.3. pH

The initial and final pH was measured with JENWAY 3505 digital pH meter.

N.B: all experiments were repeated three times, and the reported data were averages.

RESULTS AND DISCUSSION

Total carotenoids produced by the five *Rhodotorula* strains, using glucose as a sole carbon source were listed in **Table 1**. Results show that *Rhodotorula glutinis* NRRL Y-842 yielded the highest value (2328.29µg/L) at 96hr. On the other hand, the highest values obtained by *Rhodotorula armeniaca* NRRL Y-17201, *Rhodotorula mucilaginosa* NRRL Y-844, *Rhodotorula bogoriensis* NRRL Y-59880 and *Rhodotorula aurantiaca* NRRL Y-1984 were 10.99,10.99,71.13 and 62.73 µg/L at 96hr., 96hr., 120hr. and 96hr., respectively. Also, data show that the highest values of consumed sugars (gm/Liter) and carotenoids produced (µg) per gm consumed sugar were obtained by *Rhodotorula glutinis* NRRL Y-842, being 24.734 g/L and 94.133µg/g, respectively. It could be stated that, *Rhodotorula glutinis* NRRL Y-842 proved to be the best strain for carotenoids production. So, other sugars were used to study their effect on carotenoids production by this yeast strain.

Data in Table 2 show the effect of some sugars used as a carbon source, namely, sucrose, lactose, maltose and fructose on carotenoids production by *Rhodotorula glutinis* NRRL Y-842. Among the used sugars, fructose recorded the highest value of carotenoids 2166.01µg/L at 96hr., while sucrose, lactose and maltose recorded 732.45, 488.88 and 1704.97 µg/L at 72hr., 72hr. and 96hr., respectively. Also, by using fructose as a

carbon source, *Rhodotorula glutinis* NRRL Y-842 recorded the highest level of cell dry weight (11.712 g/L) and carotene / cell dry weight (184.94 µg/gm).

Data in Tables 3a&b show the effect of using some agro-industrial by-products on carotenoids production by *Rhodotorula glutinis* NRRL Y-842. Data reveal that the highest values of carotenoids were recorded with using untreated sugarcane molasses (1772.19µg/L at 120 hr.), untreated beet molasses (1571.4 µg/L at 144 hr.) and treated sugarcane molasses (1002.01µg/L. at 120 hr.). β-Carotene accumulation in yeast increased when mineral-rich molasses was supplied (Bhosale and Gadre, 2001a).

Table 1: Production of total carotenoids by yeast strains of *Rhodotorula spp.* grown on media containing glucose as a carbon source

Strain	Time	Cell dry weight (gm/liter)	Carotenoids (µg/liter)	Carotene/cell dry weight (µg/gm)	Final pH	Consumed sugars (gm/liter)	Carotene / Consumed Sugar (µg/gm)
Rhodotorula glutinis NRRL Y-842	24hr.	00.6600	0050.44	076.42	5.92	12.121	004.161
	48hr.	07.1880	0757.47	105.38	5.89	24.071	031.468
	72hr.	09.7120	1358.39	139.87	5.76	24.693	055.011
	96hr.	11.9620	2328.29	194.64	6.21	24.734	094.133
	120hr.	08.2900	1038.87	125.32	6.40	24.782	041.920
	144hr.	04.7160	0642.92	136.33	7.60	24.791	025.933
Rhodotorula armeniaca NRRL Y-17201	24hr.	00.0400	06.47	161.75	5.88	01.038	006.233
	48hr.	00.2000	06.47	032.35	5.80	01.447	004.471
	72hr.	00.2200	09.70	044.09	5.78	01.666	005.822
	96hr.	00.9800	10.99	011.21	5.67	03.176	003.460
	120hr.	00.1000	02.26	022.60	5.85	03.396	000.665
	144hr.	00.0600	02.26	037.67	5.75	03.491	000.647
Rhodotorula mucilaginosa NRRL Y-844	24hr.	00.4333	06.47	014.93	5.89	03.239	001.998
	48hr.	00.8467	06.47	007.64	5.82	03.522	001.837
	72hr.	04.8067	09.70	002.02	5.74	04.182	002.319
	96hr.	07.1333	10.99	001.54	6.08	04.434	002.479
	120hr.	03.9467	02.26	000.57	5.84	04.654	000.486
	144hr.	01.6400	02.26	001.38	6.02	05.629	000.401
Rhodotorula bogoriensis NRRL Y-5980	24hr.	00.0133	04.20	315.79	5.82	00.943	004.454
	48hr.	00.8600	06.47	007.52	6.50	03.176	002.037
	72hr.	01.4533	15.20	010.46	6.67	03.585	004.240
	96hr.	01.8133	34.60	019.08	6.96	03.742	009.246
	120hr.	07.5333	71.13	009.44	5.08	08.648	008.225
	144hr.	05.2800	29.10	005.51	5.14	09.591	003.034
Rhodotorula aurantiaca NRRL Y-1584	24hr.	00.6000	02.26	003.77	4.78	00.880	002.568
	48hr.	00.7000	06.47	009.24	4.28	01.038	006.233
	72hr.	00.7267	09.70	013.35	4.22	01.289	007.525
	96hr.	00.8667	62.73	072.38	4.20	02.138	029.340
	120hr.	00.5933	19.40	032.70	4.26	02.767	007.011
	144hr.	00.4267	04.20	009.84	4.22	03.491	001.203

initial pH= 6, at 30° C

Thus cheap, available nutrient sources such as sugarcane and beet molasses can be used to make the process of pigment production industrially

feasible. The highest production recorded by using glucose syrup (1831.36µg/L) may be attributed to the nature of glucose syrup. Glucose syrup has unique sugar content, that it contains monosaccharides, disaccharides, tetrasaccharides, pentasaccharides, hexasaccharides and high sugars (Johnson and Schroeder, 1995).

Table 2: Effect of some sugars on carotenoids production by *Rhodotorula glutinis* NRRL Y-842

Carbon source	Time	Cell dry weight (gm/litre)	Carotenoids (µg/litre)	Carotene/cell dry weight (µg/gm)	Final pH	Consumed sugars (gm/liter)	Carotene / Consumed Sugar (µg/gm)
Sucrose	24hr.	01.6580	0164.90	099.46	6.36	22.825	007.225
	48hr.	02.3440	0224.94	095.96	8.07	23.039	009.763
	72hr.	05.8000	0732.45	126.29	6.55	23.359	031.356
	96hr.	03.4040	0501.39	147.29	7.95	23.611	021.235
	120hr.	02.2572	0253.27	112.21	6.14	24.000	010.553
	144hr.	01.5360	0031.62	020.59	8.44	24.175	001.308
Lactose	24hr.	01.2680	0140.26	110.62	6.16	02.699	051.967
	48hr.	02.3600	0420.40	178.14	7.84	05.619	074.818
	72hr.	03.3740	0488.88	144.90	8.04	05.885	083.072
	96hr.	02.9020	0309.33	106.60	7.91	06.328	048.883
	120hr.	02.6880	0164.90	061.35	8.08	07.390	022.314
	144hr.	02.4140	0053.16	022.02	4.62	07.655	006.944
Maltose	24hr.	01.4020	0145.50	103.78	6.18	16.667	008.730
	48hr.	04.0120	0421.08	104.96	6.91	20.345	020.697
	72hr.	07.9900	0520.02	065.08	6.28	23.776	021.871
	96hr.	10.0600	1704.97	169.48	6.22	24.830	068.666
	120hr.	07.3780	0375.49	050.89	6.22	24.851	015.110
	144hr.	03.0960	0332.32	107.34	7.35	24.894	013.349
Fructose	24hr.	01.2480	0084.39	067.62	5.78	10.806	007.810
	48hr.	06.8540	0520.89	076.00	4.70	18.472	028.200
	72hr.	10.2120	1385.16	135.64	5.24	23.953	057.828
	96hr.	11.7120	2166.01	184.94	5.98	24.670	087.799
	120hr.	11.4120	1575.28	138.04	5.96	24.682	063.823
	144hr.	10.6280	0774.06	072.83	6.34	24.801	031.211

initial pH= 6 , at 30 C

On the other hand the lowest values were noticed with treated vinasse, soy bean flour extract and maize flour extract (452.898, 426.8, 340.79 µg/L at 120, 48 and 48 hr.), respectively. Buzzini and Martini (1999) obtained 4.74 and 3.82 mg/L carotenoids, at 120 hr., from *R. glutinis* DBVPG 6439 cultivated on a medium containing SFE and MFE, respectively. As can be seen, untreated molasses and vinasse promoted the highest amounts of carotenoids while treated molasses and vinasse recorded small amounts of carotenoids in comparable with untreated molasses and vinasse. It can be assumed that metal salts in untreated molasses have a stimulatory effect on carotenoids production and this positive effect is due to a stimulatory effect of cations on carotenoid- synthesizing system. Similar findings were reported by Bhosale and Gadre (2001a).

Table 3a: Production of carotenoids by *Rhodotorula glutinis* NRRL Y-842 using some agro-industrial raw materials

Carbon source	Time	Cell dry weight (gm/litre)	Carotenoids (µg/litre)	Carotene/cell dry weight (µg/gm)	Final pH	Consumed sugars (gm/liter)	Carotene / Consumed Sugar (µg/gm)
Beet molasses (Treated)	24hr.	1.204	0066.93	055.59	5.48	05.675	011.794
	48hr.	2.176	0267.72	123.03	5.52	05.889	045.461
	72hr.	2.540	0446.20	175.67	5.57	06.156	072.482
	96hr.	2.708	0679.00	250.74	5.71	09.529	071.256
	120hr.	2.304	0576.18	250.08	4.84	10.225	056.350
	144hr.	1.172	0261.90	223.46	5.70	11.724	022.339
Beet molasses (Untreated)	24hr.	1.824	0037.83	020.74	5.22	00.236	160.297
	48hr.	3.666	0439.41	119.86	5.59	02.305	190.633
	72hr.	4.558	0962.24	211.11	5.59	04.728	203.520
	96hr.	4.782	0993.28	207.71	5.73	05.851	169.762
	120hr.	7.218	1067.00	147.82	5.43	14.835	071.925
	144hr.	16.726	1571.40	093.95	4.60	18.558	084.675
Sugar cane molasses (Treated)	24hr.	1.694	0061.983	036.59	5.61	09.798	006.326
	48hr.	2.414	0214.855	089.00	5.53	15.994	013.433
	72hr.	2.580	0239.590	092.86	5.81	18.227	013.145
	96hr.	3.502	0543.200	155.11	5.78	18.444	029.451
	120hr.	4.192	1002.010	239.03	5.63	22.190	045.156
	144hr.	2.322	0649.900	279.89	5.68	22.334	029.099
Sugar cane molasses (Untreated)	24hr.	2.082	0083.517	040.11	5.07	05.075	016.457
	48hr.	4.702	0491.790	104.59	5.05	15.288	032.168
	72hr.	5.252	0595.483	113.38	5.88	17.293	034.435
	96hr.	6.750	1681.980	249.18	5.57	19.048	088.302
	120hr.	7.316	1772.190	242.23	5.26	20.928	084.680
	144hr.	6.672	1488.465	223.09	5.02	21.679	068.659
Vinasse (Treated)	24hr.	2.758	0071.295	025.85	5.14	01.196	059.611
	48hr.	2.968	0115.430	038.89	4.94	01.994	057.889
	72hr.	3.176	0160.050	050.39	4.94	03.657	043.765
	96hr.	3.422	0373.450	109.13	4.95	03.790	098.536
	120hr.	3.620	0452.893	125.11	4.94	04.388	103.212
	144hr.	2.726	0333.195	122.23	4.94	06.250	053.311
Vinasse (Untreated)	24hr.	2.994	0105.245	035.15	5.19	01.475	071.353
	48hr.	3.192	0124.645	039.05	5.37	02.212	056.349
	72hr.	3.534	0151.417	042.85	5.38	02.507	060.398
	96hr.	4.720	0599.557	127.02	6.68	05.383	111.380
	120hr.	8.322	0916.650	110.15	5.35	06.785	135.100
	144hr.	3.358	0188.083	056.01	5.97	07.374	025.506

initial pH= 6 , at 30° C

Table 3b: Effect of glucose syrup, soy bean flour extract (SFE) and maize flour extract (MFE) on carotenoids production by *Rhodotorula glutinis* NRRL Y-842

Carbon source	Time	Cell dry weight (gm/litre)	Carotenoids (µg/litre)	Carotene/cell dry weight (µg/gm)	Final pH	Consumed sugars (gm/liter)	Carotene / Consumed Sugar (µg/gm)
Glucose syrup	24hr.	01.224	0087.30	071.32	5.85	01.178	074.109
	48hr.	04.746	0419.04	088.29	6.83	02.462	170.203
	72hr.	07.044	0465.60	066.10	6.05	10.011	046.509
	96hr.	11.248	1831.36	162.85	5.71	14.133	129.580
	120hr.	10.310	1431.72	138.87	5.68	14.186	100.925
	144hr.	09.060	1117.44	123.34	5.55	17.720	063.061
SFE	24hr.	3.3067	247.67	74.90	6.05	01.387	178.565
	48hr.	6.1000	426.80	69.97	6.96	11.891	035.893
	72hr.	7.3067	267.07	36.55	7.43	17.941	014.886
	96hr.	8.9667	254.14	28.34	7.99	20.883	012.170
	120hr.	9.1800	232.80	25.36	9.53	21.555	010.800
	144hr.	7.7933	132.89	17.05	9.23	22.058	006.025
MFE	24hr.	3.5000	224.39	64.11	6.07	00.379	592.058
	48hr.	6.2533	340.79	54.50	6.12	03.227	105.606
	72hr.	6.9400	069.19	09.97	5.98	07.278	009.507
	96hr.	7.1733	060.14	08.38	6.06	14.050	004.280
	120hr.	7.3400	060.14	08.19	6.07	18.481	003.254
	144hr.	6.8067	053.67	07.88	6.07	19.177	002.799

initial pH= 6 , at 30° C

Data show differences in dry cell concentration and carotenoids production which may be due to composition, sugar, amino acids, vitamins, trace elements types and contents of the growth media, especially when agro-industrial by-products were used. Similar results were obtainted by Aksu and Tuğba Eren (2007).

Data reveal that, high amounts of sugars were consumed by *R. glutinis* NRRL Y- 842with the used carbon sources except in case of lactose, treated and untreated vinasse, where the consumed sugars were very low.

The pH of fermentation media increased during the 6 days of fermentation from an initial pH 6 to 9.23, 8.44 and 7.35 in soy flour extract, sucrose and maltose treatments, respectively. It decreased from 6 to 4.6 and 4.62 in untreated beet molasses and lactose treatments, respectively. Data reveal that maximum carotenoid production seemed to occur after the exponential phase. Results are in a good agreement with some previous reports (Buzzini and Martini 1999 and Frengova *et al.* 1994) and divergently from other (Shih and Hang 1996).

In general, data reveal that carotenoids production is affected by *Rhodotorula* strain and carbon source. Results are in accordance with those reported by Buzzini and Martini (1999), who found that carotenoid production was affected by *Rhodotorula* strain and carbon source.

Regarding to the effect of time course on carotenoid production by *R. glutinis* NRRL-Y-842, it could be noticed that it gradually increased during the fermentation period, tell reaching the maximum value after 72, 96, 120 or 144 hr. according to the used carbon source. Gamal *et al.*, (2007) reported that

the maximum carotenoids production by *Rhodotorula glutinis* 32 obtained after 72hrs. using bioreactor.

In conclusion, it could be stated that the maximum values of carotenoids produced by *R. glutinis* NRRL-Y-842 were obtained using glucose (2328.29µg/L at 96 hr.), fructose (2166.01 µg/L at 96 hr.), glucose syrup (1831.36 µg/L at 96 hr.), untreated sugarcane molasses (1772.19 µg/L at 120 hr.), maltose (1704.97 µg/L at 96 hr.) and untreated beet molasses (1571.40 µg/L at 144 hr.).

The capability of *R. glutinis* NRRL-Y-842 for growing on a variety of carbon sources and agro-industrial by-products is a remarkable advantage. Data indicated that *R. glutinis* will be one of the most promising yeast for the commercial production of carotenoids by the use of agricultural by-products as cheap carbon sources. So, carotenoid productivity by *R. glutinis* is also favorable and worthwhile.

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تأثير بعض السكريات ومخلفات الصناعات الزراعية على إنتاج الكاروتينات من بعض سلالات خميرة الرودوتوريولا

عبد الله العوضى إبراهيم سليم و محمد منصور قاسم وسامية محمد مرسى بيومى ومحمود محمد عوض الله السواح ودينا فتحى إسماعيل على

قسم الميكروبيولوجيا الزراعية – كلية ازراعة – جامعة المنصورة – المنصورة – مصر

أجرى هذا البحث لدراسة أكفأ السلالات الخمسة من خميرة الرودوتوريولا وهى *Rhodotorula glutinis* NRRL Y-842 و *R. armeniaca* NRRL Y-17201 و *R. mucilaginosa* NRRL Y-844 و *R. bogoriensis* NRRL Y-5980 و *R. aurantiaca* NRRL Y-1584 فى إنتاج الكاروتينات من بعض السكريات وكذلك مخلفات بعض الصناعات الزراعية .

- فقد تم تنمية السلالات الخمس فى وجود الجلوكوزبمعدل ٢٥جم/لتر كمصدر للكربون وتم التحضين على ٣٠°م فى حضان هزاز لمدة ٦ أيام وكانت أعلى القيم المتحققة من إنتاج الكاروتينات هى :

٢٣٢٨,٢٩ ميكروجرام/لتر بعد ٩٦ ساعة و١٠,٩٩ ميكروجرام/لتر بعد ٩٦ ساعة و١٠,٩٩ ميكروجرام/لتر بعد ٩٦ ساعة و ٧١,١٣ ميكروجرام/لتر بعد ١٢٠ ساعة و٦٢,٧٣ ميكروجرام/لتر بعد ٩٦ ساعة على التوالى. وعلى ذلك تكون سلالة *R. glutinis* NRRL Y-842 هى أكفأ السلالات المستخدمة.

- كما أجريت تجربة لمدة ستة أيام لإنتاج الكاروتينات من خميرة *R. glutinis* NRRL Y-842 واستخدمت فيها أربعة سكريات وتحققت أعلى معدلات الإنتاج مع كل سكر كما يلى :

سكروز (٧٣٢,٤٥ ميكروجرام/لتر بعد ٧٢ساعة) ، لاكتوز (٤٨٨,٨٨ ميكروجرام/لتر بعد ٧٢ساعة) ، مالتوز (١٧٠٤,٩٧ ميكروجرام/لتر بعد ٩٦ساعة) ، فركتوز (٢١٦٦,٠١ ميكروجرام/لتر بعد ٩٦ساعة).

- كذلك أجريت تجربة لإنتاج الكاروتينات من *R. glutinis* NRRL Y-842 باستخدام بعض نواتج الصناعات الزراعية الثانوية وتحققت أعلى معدلات الإنتاج كما يلى :

مولاس القصب المعامل (١٠٠٢,٠١ ميكروجرام/لتر بعد ١٢٠ساعة) ، مولاس القصب غير المعامل (١٧٧٢,١٩ ميكروجرام/لتر بعد ١٢٠ساعة) ، مولاس البنجر المعامل (٦٧٩,٠٠ ميكروجرام/لتر بعد ٩٦ساعة) ، مولاس البنجر غير المعامل (١٥٧١,٤٠ ميكروجرام/لتر بعد ١٤٤ساعة) ، الفيناس المعامل (٤٥٢,٨٩ ميكروجرام/لتر بعد ١٢٠ساعة) ، الفيناس غير المعامل (٩١٦,٦٥ ميكروجرام/لتر بعد ١٢٠ساعة) ، مستخلص دقيق فول الصويا (٤٢٦,٨٠ ميكروجرام/لتر بعد ٤٨ساعة) ، مستخلص دقيق الذرة (٣٤٠,٧٩ ميكروجرام/لتر بعد ٤٨ساعة) ، شراب الجلوكوز (١٨٣١,٣٦ ميكروجرام/لتر بعد ٩٦ساعة).

يتضح من النتائج أن أفضل السكريات للإنتاج هو سكر الجلوكوز وسكر الفركتوز وأفضل المخلفات الثانوية للصناعات الزراعية هى شراب الجلوكوز ومولاس القصب غير المعامل ثم مولاس البنجر غير المعامل. وتنصح الدراسة باستخدام المولاس سواء من القصب أو البنجر وذلك لرخص الثمن علاوة على المساهمة فى الحد من تلوث البيئة.

قام بتحكيم البحث

أ.د / محمود عبد المنعم خفاجى — **كلية الزراعة – جامعة المنصورة**

أ.د / حسين عبد الله الفضالى — **كلية الزراعة – جامعة دمياط**