

## Original Research Article

### Using of some agro-industrial wastes for improving carotenoids production from yeast *Rhodotorula glutinis* 32 and bacteria *Erwinia uredovora* DSMZ 30080

#### ABSTRACT

Some agro-industrial wastes such as clarified cane molasses, high test molasses, sweet whey, potato starch and corn steep liquor were tested as carbon sources or nitrogen source for growth and carotenoid accumulation using bacteria *Erwinia uredovora* DSMZ 30080 and yeast *Rhodotorula glutinis* number 32. Erlenmeyer flasks containing 100ml of production media, the flasks were inoculated with 1ml of standard inoculum and incubated at 150rpm for 4 days at 30°C. Samples were collected periodical every 24h, cell dry weight and carotenoids concentration were determined. sweet whey and high test molasses gave the highest growth being 2.85 and 7.34g<sup>l</sup><sup>-1</sup>, respectively and scored the same layout on carotenoids conc. which reach the peak during stationary phase (72h of fermentation). Using of high test molasses and sweet whey as carotenoid production media were incremented carotenoid conc. about 1.7 and 2fold (with respect to reference media). Increasing high test molasses conc. to give 5% initial sugar led to up great growth, carotenoids conc., productivity, yield and Y<sub>c/x</sub> from *Rhodo. glutinis*32 to be 7.31g<sup>l</sup><sup>-1</sup>, 2.67mg<sup>l</sup><sup>-1</sup>, 0.037 mg<sup>l</sup><sup>-1</sup>h<sup>-1</sup>, 0.067% and 0.365, respectively. Furthermore, the addition of corn steep liquor (30%) as nitrogen sources augmented carotenoids concentration about 3.8 and 4fold for incomplete and complete production media using *Rhodo. glutinis*32 while corn steep liquor have negligible effect on *E. uredovora* DSMZ 30080. Also, the same negligible effect on growth was observed with dark incubation with both strains which dropped about 75 and 48% with regard to control for *E. uredovora* DSMZ 30080 and *Rhodo. glutinis* 32, respectively, whereas, carotenoids conc. was increased about 21% for *E. uredovora* DSMZ 30080 in dark condition.

**Keywords:** carotenoids production, *Rhodotorula glutinis* 32, *Erwinia uredovora* DSMZ 30080, high test molasses, sweet whey

## 1. INTRODUCTION

Carotenoids are one of pigments which particulate solids that released into a medium without significant interaction [1]. Carotenoids have many applications in food process, cosmetics, paints, pharmaceuticals, textiles, glass etc. [2]. As well as their health benefits they act as vitamin A precursors, antioxidant, enhancers of in vitro antibody production and antitumor [3,4].

Several microorganisms, including bacteria, algae, molds and yeasts specially basidiomycetes are able to accumulate such as  $\beta$  and  $\gamma$  carotenoids, these yeasts belonged to the genera *Rhodotorula*, *Rhodospiridium*, *Sporobolomyces* and *Phaffia* [5]. Carotenoids accumulation within the yeast cell affect with different environmental and nutritional factors specially media components, their able to produced carotenoids when cultivated in commercial medium, containing various refined carbon sources, such as glucose, xylose, cellobiose, sucrose, glycerol and sorbitol nevertheless these types of medium represents high costs. Therefore, there have been a growing interest to used agro-industrial wastes as carbon sources to reduce the cost of production [6].

*Rhodotorula glutinis* NRRLY-842 recommended to be the best strain for carotenoids production, 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 [7].

As a result of stringent rules and regulations applied to chemically synthesized/purified pigments, consumer preferences and growth of food industry, demand of carotenoids as safe and suitable coloring agents is on rise. So, the need to use bacteria as carotenoids source was greater, because they have unicellular and high growth rate as well as low cost propagation media [8].

Other bacteria such as *Mycobacterium breviciae*, *Mycobacterium lacticola*, *Rhodobacter sphaeroides*, *Rhodococcus maris*, *Streptomyces chrestomycticus* and *Erwinia uredovora* also have the ability to synthesize carotenoids [9]. Biotechnologists have worked to develop number of carotenogenic genes strains which cloned from microorganisms, most of the carotenogenic genes employed in recombinant biosynthesis are derived either from *Rhodobacter* or *Erwinia species* [10]. So, this study, was carried out to use some agro-industrial wasted as carbon source or carotenoids production media for the production by yeast strain *R. glutinis* or bacteria *Erwinia uredovora* in order to reduce the cost of production process. Moreover, studying the biological activity of both microorganisms with a view to maximizing carotenoid production.

## 2. MATERIAL AND METHODS

### 2.1 Microorganism used:

The tested strains namely *Rhodotorula glutinis* number 32 and *Erwinia uredovora* DSMZ 30080 were obtained from Dept. of Microbiology, Fac. of Agriculture, Ain shams Uni. Cairo, Egypt. Stock cultures were maintenance at 5° C on preservation media YM medium for the former strain and nutrient agar for latter one.

**2.2 Inoculum seed:** Standard inoculum was prepared by inoculation of conical flasks containing 100ml of YM medium or nutrient broth with a loop full of tested strains. The inoculated flasks were incubated at 30° C and 150 rpm for 24h. The content of this flasks was used as standard inoculum (1 ml containing  $1.25\text{g l}^{-1}$  dry weight).

### 2.3 Effect of some agro-industrial wastes as a carbon sources on carotenoids production.

Clarified cane molasses, high test molasses, sweet whey, potato starch and corn steep liquor were used as a carbon source at 4% total initial sugar in combination with other constituents ( $0.5\text{ KH}_2\text{PO}_4$ ,  $0.6\text{ (NH}_4)_2\text{SO}_4$ ,  $0.2\text{ MgSO}_4\cdot 7\text{H}_2\text{O}$ ,  $0.1\text{ NaCl}$ ,  $0.01\text{ FeSO}_4\cdot 7\text{H}_2\text{O}$ ,  $0.4\text{ yeast extract}$ ,  $40\text{ glucose g l}^{-1}$ ) as synthetic carotenoids production medium Shabtai and Mukmenev media [11] for yeast or bacterial propagation. The propagation was carried out in Erlenmeyer flasks (250 ml) containing 100ml of production media, the flasks were inoculated with 1ml of standard inoculum and incubated at 150rpm for 4 days at 30°C. Samples were collected periodical every 24h, cell dry weight and carotenoids concentration were determined as well as, growth and carotenoids parameters were calculated.

### 2.4 Using of some agro-industrial wastes as a whole production media.

In this experiment, the most effective wastes were used as carotenoids production media without any additives using *Rhod. glutinis* 32 or *E. uredovora* DSMZ 30080, after inoculation the production process was going as mention before.

### 2.5 Carotenoids concentration as influenced with different sugar concentration.

A series trials of sugar of the most effective wastes were used in combination with other constituents of carotenoids production medium used to evaluated the growth and carotenoids concentration accumulated within the cell of both *Rhod. glutinis* number 32 or *Erwinia uredovora* DSMZ 30080. The propagation was carried out as noticed before.

### 2.6 Effect of using corn steep liquor as a nitrogen sources in carotenoids production.

Carotenoids production media were supplemented with different concentration of corn steep liquor as nitrogen source with or without combination other constituents of production media, Elementary flasks contained 100 of production media were inoculated and incubated as reported before then the growth and carotenoid concentration were determined.

### 2.7 Effect of light and dark on carotenoids production.

Erlenmeyer flasks (250 ml) containing 100ml production media containing the most efficient carbon and nitrogen sources, after inoculation were incubated at light (at intensity of illumination about 60W) or in dark condition for 72h at 30°C. At the end of fermentation period, the parameters of growth and carotenoid production were detected.

## 2.8 Carotenoids extraction and determination:

Carotenoid pigments were extracted from the cells and determined according to the method described by Frengova *et al* [12].

## 2.9 growth and carotenoids parameters:

### 2.9.1 The specific growth rate ( $\mu$ ) and doubling time ( $t_d$ ).

The specific growth rate ( $\mu$ ) and doubling time ( $t_d$ ) were calculated from the exponential phase according to Painter & Marr [13] using the following equation:-

$$\mu = (\ln A - \ln A_0) t^{-1}$$

$$t_d = \ln 2 \cdot \mu^{-1} \text{ where}$$

$\mu$  = Specific growth rate ( $h^{-1}$ )

A = Amount of growth after t time

$A_0$  = Amount of growth at the beginning of logarithmic phase

t = Time of the logarithmic phase,  $t_d$  = Doubling time

### 2.9.2. Number of generations (N).

Number of generations was calculated using the following equation according to Stanier *et al* [14]:

$$N = t/t_d$$

where

N = Number of generations

t = the period of exponential (h)

$t_d$  = doubling time (h)

### 2.9.3 Multiplication rate (MR) [14].

Multiplication rate was calculated according to the following equation:

$$MR = N t^{-1} \text{ Where:}$$

MR = Multiplication rate

N = Number of generations

t = Time of the logarithmic phase

### 2.9.4 Carotenoids yield (%) = gram carotenoids X 100/gram original sugar, according to Ramadan *et al* [15]

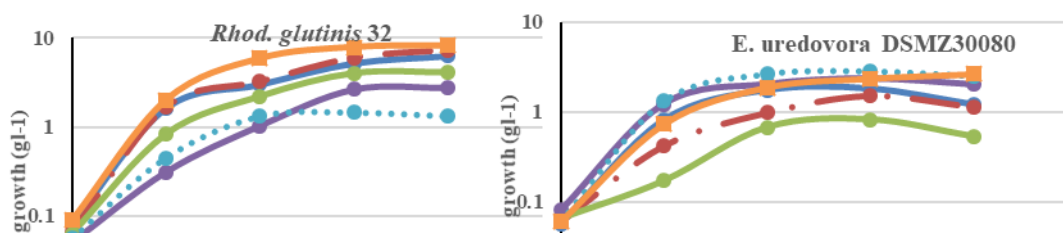
### 2.9.5 Carotenoids yield coefficient relative to biomass ( $Y_{c/x}$ ) = gm carotenoids/ gm biomass dry weight according to Grothe *et al* [16].

### 2.9.6 Carotenoids productivity = mg carotenoids / fermentation time (h) $mg l^{-1} h^{-1}$ , according to Lee [17].

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of some agro-industrial wastes as a carbon sources in production media.

In this experiment the growth and carotenoid conc. were evaluated using carotenoid production media as influenced with different agro-industrial wastes as a carbon sources, data were illustrated in Fig. (1). The growth of both *Rhod. glutinis* 32 and *Erwinia uredovora* DSMZ30080 were increased gradually with fermentation time to reach the maximum after 3 or 4 days of incubation period using sweet whey and high test molasses as carbon sources being 2.85 and 7.34  $g l^{-1}$  for *E. uredovora* DSMZ30080 and *Rhod. glutinis* 32, respectively. On the other hand, corn steep liquor and sweet whey reduced the growth of both *E. uredovora* DSMZ30080 and *Rhod. glutinis* 32 about 95 and 87.85%, respectively, with respect to synthetic production medium. All tested agro-industrial wastes supported the exponential growth of both *E. uredovora* DSMZ30080 and *Rhodo. glutinis* 32 during the first 48h except potato starch waste which reduce the exponential growth about 24h for the former strain and prolonged the log phase to reach 72h for the latter one. Moreover, potato starch waste gave the highest specific growth rate and multiplication rate as well as lowest doubling time being 0.113  $h^{-1}$ , 0.163 & 6.13h for *E. uredovora* DSMZ30080. The corresponding figures obtained by *Rhodo. glutinis* 32 on high test molasses being 0.081  $h^{-1}$ , 0.117 & 8.545h, respectively. On the contrary, potato starch waste and corn steep liquor gave the lowest growth parameters for *Rhodo. glutinis* 32 and *E. uredovora* DSMZ30080.



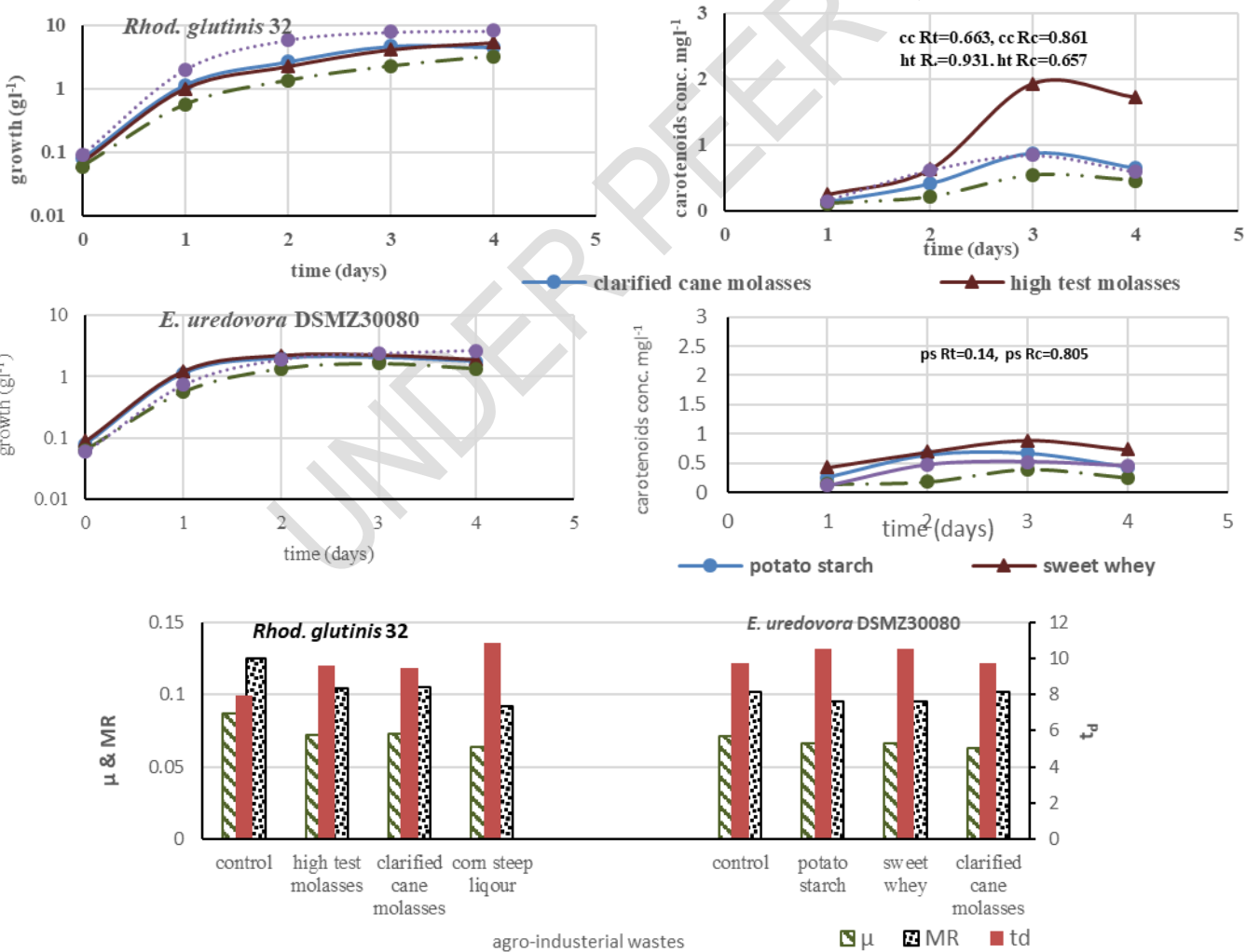
**Fig. 1. Growth, carotenoids concentration and growth parameters on carotenoid production media as influenced with different agro-industrial wastes as carbon sources.**

Carotenoids concentration increased gradually with fermentation time to reach the peak after 72h of fermentation (the stationary phase) for both *Rhodo. glutinis* 32 and *E. uredovora* DSMZ30080. These results are in accordance with those *Selim et al* [7], they found that carotenoids concentration reaching the maximum value after 72, 96, 120 or 144h. High test molasses induced carotenoids production by *Rhodo. glutinis* 32 to give the highest values of carotenoids concentration, yield and productivity being 2.45 mg<sup>l</sup><sup>-1</sup>, 0.0061mgg<sup>-1</sup> & 0.034 mg<sup>l</sup><sup>-1</sup>h<sup>-1</sup>, which increased about 2.5, 2.9, 3.7 & 2.9fold comparing to synthetic production medium. *Selim et al* [7] reported the same results with un-clarified cane molasses. Generally, *El-Banna et al* [18] found that the either of high fructose corn syrup or glucose resulted in production of higher percentage of β-carotene from *Rhodotorula glutinis* var. *glutinis*. Both sucrose or glucose syrup gave higher percentage of torulene and torularhodin. Sweet whey, has the same effect on carotenoids from *E. uredovora* DSMZ30080 which up grad carotenoids concentration about 2fold, while adding corn steep liquor as a carbon source in production medium decline carotenoids concentration which recorded the lowest figure being 0.34 mg<sup>l</sup><sup>-1</sup>. As well as carotenoids concentration also varied according to the agro-industrial waste which used as carbon source in production medium so, it could be ranked the agro-industrial wastes in descending order as follow: high test molasses> clarified

molasses> corn steep liquor> potato starch> sweet whey for *Rhodo. glutinis*32 and sweet whey> potato starch> clarified molasses> high test molasses> corn steep liquor for *E. uredovora* DSMZ30080.

### 3.2 Using of the most efficiency agro-industrial wastes as a carotenoids production media.

In this experiment, the most effective agro-industrial wastes were used as carotenoids production media. Data in Fig. (2) reveal that the highest growth observed after 72 and 96h of incubation which ranged from 1.62 to 2.2 and 3.21 to 5.32 for *E. uredovora* DSMZ30080 and *Rhodo. glutinis* 32, respectively. It could be noticed that reference synthetic medium still the favorites for yeast and bacterial growth, this observation may be related to the effect of the minerals and other component in original production medium on growth. All agro-industrial wastes supported exponentially growth during the first 48h of fermentation for both tested strains except potato starch waste and sweet whey which decreased the exponential growth of *E. uredovora* DSMZ30080 to 24h and scored the highest figure of specific growth rate and lowest doubling time being  $0.0108\text{h}^{-1}$ , 6.32h,  $0.109\text{h}^{-1}$  & 6.35h, respectively. Whereas, clarified cane molasses same mode followed by high test molasses for *Rhodo. glutinis* 32. The time course of carotenoids production increased to reach the peak during the first 72h of incubation (stationary phase) using sweet whey and high test molasses as production media which recorded the highest carotenoids concentration and productivity being  $0.89\text{mg l}^{-1}$ ,  $0.018\text{mg l}^{-1}\text{h}^{-1}$ ,  $1.93\text{ mg l}^{-1}$  &  $0.027\text{ mg l}^{-1}\text{h}^{-1}$  for *E. uredovora* DSMZ30080 and *Rhodo. glutinis* 32, respectively about 1.7 and 2fold incremental in carotenoids concentration (with respect to control). On contrary corn steep liquor and clarified cane molasses lower carotenoids concentration about 0.63 & 0.75fold for *Rhodo. glutinis* 32 and *E. uredovora* DSMZ30080, respectively.

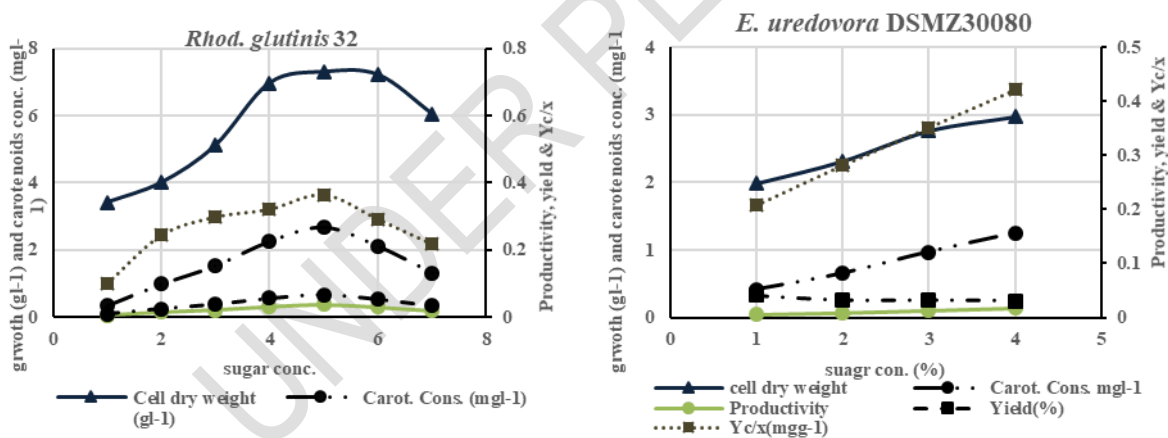


**Fig. 2. Growth, carotenoids concentration and growth parameters using some agro-industrial wastes as a carotenoids production media.** Rt= correlation coefficient of clarified cane molasses related to time, Rc= correlation coefficient of clarified cane molasses related synthetic media (control), cc=clarified cane molasses, ht= high test molasses, ps= potato starch, sw= sweet whey

These data were in line with **Selim et al** [7] found that the highest values of carotenoids production by *Rhodotorula glutinis* NRRL Y-842 were observed by using untreated sugarcane molasses followed by untreated beet molasses and treated sugarcane molasses. These results may be related to the occurrence of some minerals or trace elements as well as vitamins, amino acids and short peptides in a small amount in molasses which supported microbial growth and carotenoids production. The Stimulatory effect of trace metal ions on carotenogenesis has been explained by hypothesizing a possible activation or inhibition mechanism of carotenogenic enzymes specifically distresses involved in carotenoid biosynthesis [19].

**3.3 Effect of sugar concentrations on carotenoids production from *Rhodo. glutinis* 32 or *Erwinia uredovora* DSMZ30080.**

This experiment was designed to study the effect of different concentrations of sugar using high test molasses or sweet whey as whole medium for carotenoids production by *Rhodo. glutinis*32 and *E. uredovora* DSMZ30080. Data presented in Fig (3) show that growth of *Rhodo. glutinis*32, carotenoids concentration, productivity, yield and  $Y_{c/x}$  were increased gradually with fermentation time and recorded the highest figures being  $7.31\text{gl}^{-1}$ ,  $2.67\text{mgl}^{-1}$   $0.037$ ,  $\text{mgl}^{-1}\text{h}^{-1}$ ,  $0.067\%$  and  $0.365$ , respectively, after 72h of fermentation period (during stationary phase) on high test molasses containing 5% sugar then the growth and carotenoids



**Fig. 3. Growth and carotenoids concentration of *Rhodo. glutinis*32 or *E. uredovora* DSMZ30080 as affected with different sugar concentrations using high test molasses or sweet whey as sugar source.**

concentration was decline. Increasing the sugar concentration of high test molasses media resulted in decreasing yeast growth, carotenoid concentration as well as prolonged in log phase. While, the lowest growth and carotenoids concentration were achieved at 1% sugar being  $3.42\text{gl}^{-1}$  and  $0.34\text{mg l}^{-1}$ , respectively. **Ferrao and Garg** [20] found that the high concentration of carbon sources led to an inhibitory effect on growth and  $\beta$ -carotene accumulation of *Rhodotorula* sp.

Data presented in Fig (3) indicated that using different concentration of whey as a sugar source have the same trend on *E. uredovora* DSMZ30080 growth, carotenoids concentrations and productivity that increased gradually with whey to reach the maximum at 4% sugar being  $2.97\text{g l}^{-1}$ ,  $1.25\text{ mg l}^{-1}$  &  $0.017\text{ mg l}^{-1}\text{h}^{-1}$ , which also recorded the highest specific growth rate and multiplication rate as well as, lowest doubling time being  $0.083\text{h}^{-1}$ ,  $0.11$  &  $8.35\text{h}$ , respectively (data not show).

### 3.4 Effect of using corn steep liquor as nitrogen source in carotenoids production.

Corn steep liquor conc.%	Production media	Cell dry weight ( $\text{g l}^{-1}$ )	Carot. Cons. ( $\text{mg l}^{-1}$ )	Carotenoids parameters		
				Productivity ( $\text{mg l}^{-1}\text{h}^{-1}$ )	Yield (%)	$Y_{c/x}$ ( $\text{mg g}^{-1}$ )
0 (control)	Complete m.	6.75	$2.12 \pm 0.01$	0.029	0.0053	0.314
10		4.42	$1.45 \pm 0.01$	0.020	0.0036	0.328

In this experiment, different corn steep liquor concentrations were used as nitrogen source in complete or incomplete molasses carotenoids production media by *Rhod. glutinis* 32. Data presented in Table (1) show that the yeast growth and carotenoids concentration were incremented as corn steep liquor concentrations raised up to 30% to give the highest values being  $6.57\text{g l}^{-1}$ ,  $2.33\text{mg l}^{-1}$  and  $6.83\text{g l}^{-1}$ ,  $2.41\text{ mg l}^{-1}$  for incomplete and complete media, respectively, at the

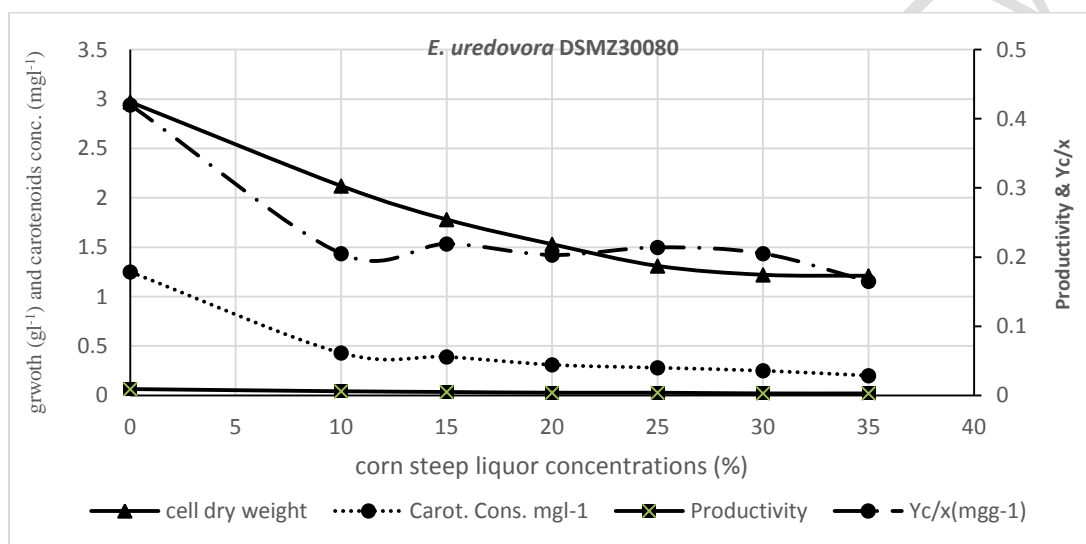
end of fermentation. Carotenoids concentrations raised about 3.8 and 4fold for incomplete and complete media, respectively with contrast to synthetic medium. Moreover, there is a slight variation in carotenoids and growth values between complete and incomplete media, it could be related to the availability of carbohydrates, protein and fibers which achieved in molasses in addition to the minerals and vitamins as well as bioactive compounds presented in high test molasses in production media [21]

Data presented in Fig (4) indicated that corn steep liquor was unfavorable nitrogen source for both growth or carotenoids production by *E. uredovora* DSMZ30080, corn steep liquor disconcert the growth, carotenoids concentration, productivity and  $Y_{c/x}$  after 72h of fermentation. On contrary, the highest specific growth rate, multiplication rate and lowest doubling time were observed at 10% corn steep liquor being  $0.042\text{h}^{-1}$ ,  $0.065$  and  $15.4\text{h}$ , respectively (data not show).

**Table 1. Growth and carotenoids concentration of *Rhodo. glutinis* 32 on complete or incomplete molasses production media as influenced by different corn steep liquor concentrations as nitrogen sources.**

15		6.12	1.83±0.03	0.025	0.0046	0.299
20		6.53	2.01±0.01	0.028	0.0050	0.308
25		6.65	2.22±0.02	0.031	0.0056	0.334
30		6.83	2.41±0.02	0.033	0.0060	0.353
35		6.78	2.38±0.03	0.033	0.0060	0.351
10	Incomplete m.	5.87	1.05±0.03	0.015	0.0026	0.179
15		6.21	1.68±0.01	0.023	0.0042	0.271
20		6.20	1.88±0.01	0.026	0.0047	0.303
25		6.43	2.01±0.01	0.028	0.0050	0.313
30		6.57	2.33±0.02	0.032	0.0058	0.355
35		6.55	2.3±0.02	0.032	0.0058	0.351

±= standard error



**Fig. 4. Growth and carotenoids concentration of *E. uredothora* DSMZ30080 as affected with different corn steep liquor used as nitrogen source after 72h of fermentation.**

### 3.5 Effect of light and dark on carotenoids production using both *Rhodo. glutinis* 32 and *E. uredothora* DSMZ30080.

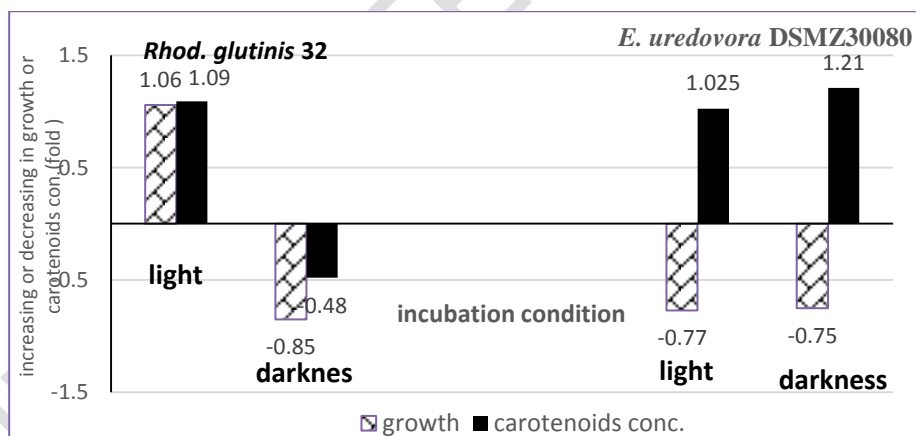
The difference between the effect of darkness and light conditions during fermentation on *Rhodo. glutinis* 32 and *E. uredothora* DSMZ30080 growth and carotenoids production were tabulated in Table (2) and Fig (5). Both strains *Rhodo. glutinis* 32 and *E. uredothora* DSMZ30080 grow exponentially during first 72h of fermentation except *E. uredothora* DSMZ30080 in dark condition which minimized the exponential phase about 24h, as well as, the lowest specific growth rate and high doubling time was obtained at dark condition for both tested strains. Time course of carotenoids production was not varied with fermentation condition (being 72h of fermentation) for both tested strains.



**Table 2. Effect of light or dark on growth and carotenoids concentration of *Rhodo. glutinis* 32 or *E. uredovora* DSMZ30080 on mixture of high test molasses and corn steep liquor or sweet whey.**

R <sub>t</sub> = coefficient fermentation	Time (days)	Tested strains	conditions	Cell dry weight (g l <sup>-1</sup> )	Carot. Cons. (mg l <sup>-1</sup> )	Carotenoids parameters			correlation related to time
						Productivity (mg l <sup>-1</sup> h <sup>-1</sup> )	Yield (%)	Yc/x (mg g <sup>-1</sup> )	
	1	<i>Rhodo. glutinis</i> 32	light	1.14	0.97±0.04	0.013	0.0024	0.851	
	2			3.24	1.87±0.04	0.026	0.0047	0.577	
	3			6.98	2.54±0.01	0.035	0.0064	0.364	
	4			6.02	2.01±0.06	0.028	0.0050	0.334	
			R <sub>t</sub>	0.868	0.879884				
	1		dark	1.1	0.31±0.02	0.004	0.0008	0.282	
	2			2.04	0.85±0.02	0.012	0.0021	0.417	
	3			5.12	1.12±0.03	0.016	0.0028	0.219	
	4	4.64		0.98±0.02	0.014	0.0025	0.211		
		R <sub>t</sub>	0.875	0.783					
		1	<i>E. uredovora</i> DSMZ30080	light	0.75	0.31±0.01	0.004	0.0008	0.413
		2			1.89	0.76±0.01	0.011	0.0019	0.402
3		2.21			1.23±0.03	0.017	0.0031	0.557	
4		2.01			0.72±0.02	0.010	0.0018	0.358	
		R <sub>t</sub>		0.824	0.780				
1		dark		0.98	0.31±0.05	0.004	0.0008	0.316	
2				2.01	0.98±0.04	0.014	0.0025	0.488	
3				2.15	1.46±0.02	0.020	0.0037	0.679	
4	2.53		1.01±0.02	0.014	0.0025	0.399			
	R <sub>t</sub>	0.570	0.834						

±=standard error



**Fig. 5. Increasing or decreasing in growth and carotenoids conc. of *Rhodo. glutinis* 32 or *E. uredovora* DSMZ30080 incubated under light or dark condition.**

Light condition incremented carotenoid concentration, content and productivity of *Rhodo. glutinis* 32 which raised more than 2fold than darkness. The absence of light during fermentation led to negligible effect on carotenoids concentration which dropped about 48% with regard to control. On the other hand, dark condition has the same carotenoids layout for *E. uredovora* DSMZ30080 to give 1.46mg l<sup>-1</sup>, 0.068%, 0.02mg l<sup>-1</sup> h<sup>-1</sup> & 0.0037, respectively, which give about 21% increasing in carotenoid concentration. This data was in line with **Stachowiak and Czarnecki** [22] which reported that light stimulated carotenogenesis in cells of the *Phaffia rhodozyma* CBS 5626 yeast. The highest yields of carotenoids

were obtained in cultures run at constant illuminance of 400 lux and 600 lux, while the lowest in culture run in the dark. Moreover, **Khodaiyan et al** [23] stated that cultivation of the bacterium *Dietzia natronolimnaea* in the absence of light decreased its cell growth and pigment formation. In addition, there were significant decreases in the biomass, carotenoids, and canthaxanthin production.

#### 4. CONCLUSION

In this investigation, we try to use some agro-industrial wastes as a production media, carbon or nitrogen sources for carotenoids production from yeast *Rhod. glutinis* 32 and bacteria *E. uredovora* DSMZ30080. Thus, effective use of cheaply available agro-industrial residues for the production of microbial pigments can make the process cost effective and environment friendly [21]. Data revealed that, high test molasses, clarified cane molasses and corn steep liquor were the favorable carbon source for growth and carotenoid production of yeast strains while sweet whey, potato starch and clarified cane molasses have the same mode with the bacteria *E. uredovora* DSMZ30080, these observation supports the use of this waste as a production medium without any additives resulted in inducing the carotenoids cons. about 1.7 and 2fold from bacteria and yeast, respectively. Moreover, increasing sugar concentration to 5% in high test molasses medium supported the carotenoids production from *Rhod. glutinis* 32. With regard to nitrogen sources corn steep liquor concentration affected growth and carotenoids conc. directly to give the highest values at 30% corn steep liquor from *Rhod. glutinis* 32 growing in complete or incomplete media. Darkness condition has a negligible effect on the growth of both strains which dropped about 75 and 48% with regard to synthetic production medium for *E. uredovora* DSMZ 30080 and *Rhod. glutinis* 32, respectively, and increased carotenoids conc. about 21% from *E. uredovora* DSMZ 30080 in dark condition.

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