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EVALUATION OF CAROTENOIDS PRODUCED BY *RHODOTORULA GLUTINIS* USING BIOREACTOR WITH DIFFERENT FEEDING TECHNIQUES ON ALBINO RATS

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ABSTRACT

Carotenoids production was studied by cultivating *R. glutinis* 32 on modified MM-4 in bioreactor as a batch, two stage batch and fed batch cultures. Five incubated temperatures as well as five agitation speeds were tested for carotenoids production using batch bioreactor technique. The highest figures of carotenoids concentration and productivity were 4.24 mg L^{-1}, and 0.044 mg $L^{-1}h^{-1}$ respectively on MM-4 incubated at 32C, 550 rpm after 72 h using two stage batch technique, while 44% and 27% were loss respectively on molasses medium. Two feeding rates of glycerol were studied during the production of carotenoids using fed batch culture, the highest carotenoids parameters were recorded at 4.0 $gL^{-1}h^{-1}$ glycerol feeding rate. The latter technique increased the concentration, content and productivity of carotenoids by *R. glutinis* to 1.26, 1.09 and 1.26 fold respectively as compared with that obtained in batch bioreactor technique after 72 hr. The statistical analysis of animal feeding on plant, microbial carotenoids, or normal diet proved that the difference in T_3, T_4 hormones and daily gain among the three groups were insignificant. GOT and ALP enzymes as well as urea concentration in blood had no significant differences, except GPT and uric acid concentration among the three groups. The histopathological studies showed normal histological structure with no clinical carcinogenic signs either of liver or kidney rats of the three groups.

INTRODUCTION

Microbial sources are being exploited to produce natural coloring substances to avoid the toxicity of synthetic colorants. So, microbial carotenoids are becoming more important as natural colorants and ingredients for a variety of applications. Carotenoid pigments may be used as additives in the food, feedstuff, pharmaceutical or cosmetic industries **(Tsubokura *et al*, 1999).** They can also be used in agriculture to enhance flower, fruit or plant color. Carotenoids seem to play a fundamental role, their presence in the human diet being considered positively because of their action as pro-vitamin A **(Johnson and Schroeder, 1996)**, to prevent or correct of vitamin A deficiency in man, antioxidant or possible tumor-

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inhibiting agents **(Steck *et al*, 2004)** due to their ability to react as powerful antioxidant.

Several bacteria, fungi and yeasts are effective carotenoids producers. These carotenoids are natural pigments, but only beta-carotene is produced commercially on a limited scale, and at high cost, from just one microbial source. Moreover, many investigators **(Buzzini & Martini, 1999 and Buzzini, 2001)** stated that the red yeasts belonging to genus *Rhodotorula* may have industrial relevance as carotenoid producers.

Therefore, the present study was designed to study the production of carotenoids by *R. glutinis* under bioreactor conditions of either batch; two stage batch or fed-batch culture. Moreover, toxicological evaluation of carotenoids produced was studied to asses their safety in experimental animals.

MATERIALS AND METHODS

Yeast strain used

A preliminary screening program by **Galal, (2004)** for high carotenoid producer yeast, showed that *Rhosotorula glutinis* 32 was the most active on MM-4 medium.

Media used

1- Propagation medium

Modified CAIM, (1987) as recorded by **Galal, (2004),** this medium was used for yeast propagation. It consists of (gL^{-1}) Yeast extract, 10; Bacto peptone, 20; glucose, 10 and Agar, 20; pH 4.5-5.0.

2- Production medium (Galal, 2004), modified medium No. 4 (MM-4) which consists of (gL^{-1}) glycerol, 40; Ammonium nitrate, 0.111; Yeast extract, 1.033, $MgSO_4$. 7 H_2O, 0.2; KH_2PO_4, 6.25; NaCl, 0.75 and $FeSO_4$. $7H_2O$, 0.005; pH 5.0.

Standard inoculum was prepared by inoculation of conical flasks (250 ml) containing 100 ml of modified CAIM with a loop of tested strain. The inoculated flasks were incubated on shaker (150 rpm) for 24 hr at 30°C. Three ml of the culture were used as a standard inoculum (1.25 g dried cells/L) in fermenter experiments.

Bioreactor experiments

In this present work, 3 L dished bottom bioreactor 26110/Coob (Cole-Parmer Instrument) was used, which consists of 3 liter vessel equipped with lipseal stirrer assembly, automatic pH controller, automatic dissolved O_2, automatic temperature controller, foam controller and multichannel peristaltic pump (for feeding): *R. glutinis* was grown in the bioreactor as batch, two sage batch and fed-batch cultivation. .

1. In batch culture

The fermentation vessel containing 1940 ml production medium was autoclaved at 121°C for 20 min, then inoculated with 3% standard inoculum to give 2 liter working volume. Initial pH was adjusted to 5±0.1. Initial dissolved O_2 was kept at 20% saturation. Effect of incubated temperature and aeration rate in carotenoids production were investigated by using different temperatures ranged form 25 to 34 °C and different agitation speed ranged from 150 to 750 rpm. Samples (10-20ml) were taken every 3-12 hours for microbiological and chemical analyses.

2. In two stage batch culture

The first stage was carried out by inoculation of 100 ml modified CAIM medium (in 250 ml conical flasks) with 3 ml (1.25g dried cells /L) standard inoculum, then incubated at 30°C for 24 h on rotary shaker (150 rpm). The cells were harvested by centrifugation, washed with sterile distilled water, presuspended in sterile productive medium and used to inoculate the fermentation vessel to give 2 L final working volume. Modified medium 4 and molasses medium were used during second stage of cultivation. Temperature, initial pH and agitation speed were kept at 32°C ± 1°C, 5.0 ± 0.1 and 550 rpm, respectively.

3. In fed-batch culture

Continuous feeding of glycerol solution (10%) was fed continuously during the first 10 or 30 hrs of fermentation period at rate of 4 or 1.33 g $L^{-1}h^{-1}$. respectively. The operating condition and propagation as well as microbilogical or chemical analyses were done as mentioned before.

Experimental analysis

Fifteen mature male albino rats were randomly divided into three groups with five animals in each group and housed individually in group cages. The rats were weighed and fed the basal daily diet,

which contained white corn, beans, dried bread and fresh water. All animals were administrated orally by a stomach tube as: G1 (Control) sunflower oil (dissolving agent) only, G2 (Plant) sunflower oil with plant β-carotene. G3 (Microbial) sunflower oil with microbial carotenoids. Doses were used on the basis of daily requirements of β-carotene of rats as 2.39 mg per kilogram body weight **NRC, (1987)**. The animals were weighed and kept under observation for a period of 28 days for the symptoms of toxicity and cancer. At the end of acute period the animals were weighed and the blood was collected then the animals were sacrificed and liver and kidney were removed for histological examination.

Blood samples were withdrawn by heart puncture to determine some blood parameters and hormones T_3 (Triiodonthyronine) and T_4 (Tetraiodothyronine) as indicators to thyroid function and their relation to the metabolic processes and daily gain. Glutamic pyruvic transaminase (GPT), Glutamic oxaloacetic transaminase (GOT).and Alkaline Phosphatase (ALP) as indicators for liver function and Urea, creatinine and uric acid as indicators for kidney function.

Chemical determination

Carotenoids extraction and determination

Carotenoid pigments were extracted from the productive yeast cells and determined according to the method described by **Frengova *et al* (1994)**.

Triiodothyronine (T_3) and Tetraiodothyronine (T_4) determination

T_3 and T_4 concentration in rats plasma or serum was carried out according to the method recommended by **Wistom (1976)**.

GPT and GOT were determined according to the method of **Schmidi & Schmidi (1963)**, whereas **ALP** was determined according to the method of **Belfielda and Goldberg (1971)**.

Urea determination: Urea concentration was assayed enzymatically using urease. In an alkaline medium, the ammonium ions react with the salicylate and hypochlorite to form a green colored indophenol according to **Patton and Crouch (1977)**.

Creatinine determination

Creatinine in alkaline solution reacts with picrate to form a colored complex. The rate of formulation of the complex was measured photometrically at 492 nm according to **Henery (1974)**.

Uric acid determination

Enzymatic color test on basis of Trinder-Reaction according to **Trinder (1969)** was applied to determine uric acid.

Histopathological examination

The rats were sacrificed and tissue specimens from liver and kidneys were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections of about 4-6 microns in thickness were prepared and stained by haematoxylin and eosin after **(Luna, 1968)**.

Statistical analysis

Afixed model was used to detect significance of temperature and time on carotenoids production **(SAS, 1989)** for Table (1), and different agitation speeds and time for Table (2). It was difficult to test first order interactions because of the insufficient subclass numbers significant differences between the levels of the factors were also detected by Duncan's multiple rang test **(Duncan, 1955)**. A simple fixed model was used to obtain results of Table (6 & 7).

Calculation of carotenoids parameter

Yield %, yield coefficient mg/g cell dry weight, content % and productivity $mgL^{-1}\ h^{-1}$ of carotenoid pigments were performed according to **Ramadan *et al* (1985); Grothe *et al* & (1999); Lee & Chol (1998) and Lee (1996)**, respectively.

RESULTS AND DISCUSSION

I. Production of carotenoid pigments by *R. glutinis* 32 using bioreacor

I.1. As a batch culture technique

Regarding to the amount of carotenoids (mg L^{-1}) produced by *R. glutinis* 32 at different incubated temperature 25-34°C **(Table 1 & Fig. 1)**, it

Table 1. Effect of incubated temperature on carotenoids production by *Rhodotorula glutinis* 32 on modified med.4 during 72 hours incubation period using bioreactor as a batch culture.

Incubated temperature	25 °C			28 °C			30 °C			32 °C			34 °C		
Time (hours)	Cell dry weight (gl^{-1})	Carotenoid		Cell dry weight (gl^{-1})	Carotenoid		Cell dry weight (gl^{-1})	Carotenoid		Cell dry weight (gl^{-1})	Carotenoid		Cell dry weight (gl^{-1})	Carotenoid	
		Cons. (mgl^{-1})	$Y_{c/x}$ (mgg^{-1})		Cons. (mgl^{-1})	$Y_{c/x}$ (mgg^{-1})		Cons. (mgl^{-1})	$Y_{c/x}$ (mgg^{-1})		Cons. (mgl^{-1})	$Y_{c/x}$ (mgg^{-1})		Cons. (mgl^{-1})	$Y_{c/x}$ (mgg^{-1})
0	0.19^{b}	-	-	0.18^{b}	-	-	0.2^{a}	-	-	0.21^{a}	-	-	0.20^{a}	0.0	0.0
3	0.33^{d}	0.039^{d}	0.118^{d}	0.31^{e}	0.042^{c}	0.135^{c}	0.36^{b}	0.054^{b}	0.15^{b}	0.365^{b}	0.114^{a}	0.312^{a}	0.39^{a}	0.0	0.0
6	0.46^{a}	0.078^{d}	0.170^{c}	0.50^{d}	0.102^{c}	0.204^{b}	0.8^{b}	0.105^{b}	0.131^{d}	0.60^{c}	0.134^{a}	0.223^{a}	1.0^{a}	0.0	0 0
9	0.55^{d}	0.122^{d}	0.222^{c}	0.69^{c}	0.155^{c}	0.225^{b}	0.82^{b}	0.185^{b}	0.226^{b}	0.81^{b}	0.217^{a}	0.268^{a}	1.0^{a}	0.0	0.0
12	0.61^{e}	0.168^{d}	0.275^{c}	0.97^{d}	0.272^{c}	0.280^{b}	1.12^{c}	0.327^{b}	0.292^{a}	1.23^{b}	0.337^{a}	0.274^{c}	1.25^{a}	0.0	0.0
24	0.66^{d}	0.207^{d}	0.314^{c}	1.25^{c}	0.397^{c}	0.318^{c}	1.32^{b}	0.442^{b}	0.335^{b}	1.27^{c}	0.502^{a}	0.395^{a}	1.46^{a}	0.016^{e}	0.011^{d}
27	0.71^{d}	0.238^{d}	0.335^{c}	1.30^{c}	0.437^{c}	0.336^{b}	1.42^{b}	0.493^{b}	0.347^{b}	1.32^{c}	0.620^{a}	0.470^{a}	1.65^{a}	0.062^{e}	0.038^{d}
30	0.81^{e}	0.275^{d}	0.340^{d}	1.35^{d}	0.497^{c}	0.368^{b}	1.54^{b}	0.642^{b}	0.417^{b}	1.39^{c}	0.758^{a}	0.545^{a}	1.85^{a}	0.064^{e}	0.035^{e}
33	0.89^{e}	0.315^{d}	0.354^{d}	1.51^{d}	0.655^{c}	0.434^{c}	$B1.65^{c}$	0.781^{b}	0.473^{b}	1.87^{b}	1.021^{a}	0.546^{a}	2.05^{a}	0.081^{e}	0.041^{e}
48	0.97^{d}	0.315^{d}	0.325^{c}	1.56^{c}	0.797^{c}	0.511^{b}	1.82^{b}	0.930^{b}	0.511^{b}	2.20^{a}	1.385^{a}	0.630^{a}	2.2^{a}	0.138^{e}	0.063^{d}
72	0.97^{e}	0.275^{d}	0.284^{d}	1.73^{d}	0.798^{c}	0.461^{c}	2.08^{c}	0.927^{b}	0.446^{b}	2.2^{b}	1.440^{a}	0.655^{a}	2.3^{a}	0.084^{e}	0.037^{e}
96	0.98^{e}	0.222^{d}	0.227^{d}	1.73^{d}	0.676^{c}	0.390^{c}	2.07^{c}	0.751^{b}	0.363^{b}	2.2^{b}	1.354^{a}	0.615^{a}	2.3^{a}	0.071^{e}	0.031^{e}

$Y_{c/x}$ = Carotenoid yield coefficient (mgg^{-1} dry cell)

Means in the same row within each classification having the different letters are significantly ($p \leq 0.001$) different.

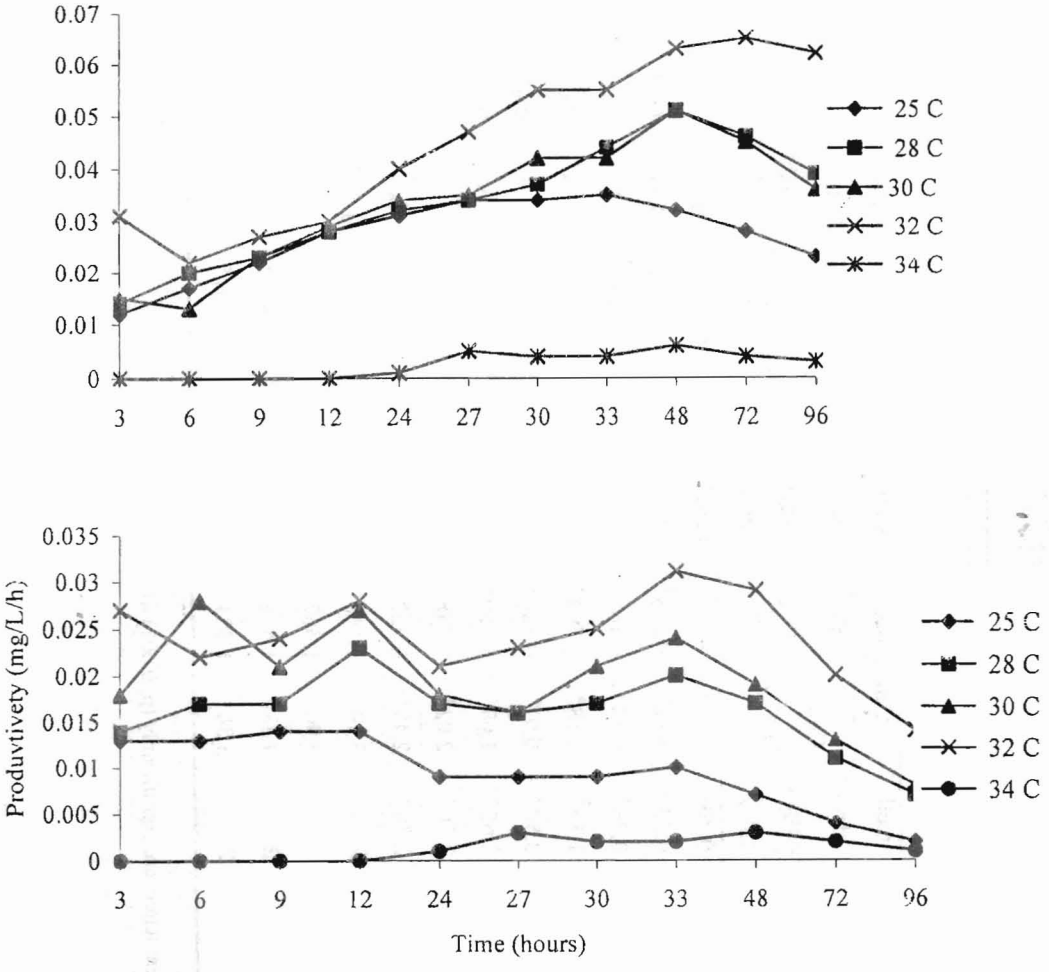

Fig. 1. Parameter of carotenoid produced by *Rhodotorula* glutinis 32 grown on modified med.4 at different incubated temperatures for 96 hrs incubation period using bioreactor as a batch culture.

could be noticed that it gradually increased during the fermentation period, till reaching the maximum at 32°C after 72 hrs. Data also revealed that carotenoids concentration, yield coefficient and content were increased significantly ($p \leq 0.001$) by increasing incubation temperature till reached the maximum being 0.655 mg g^{-1} dry weight and 1.44 mg L^{-1}, 0.065% respectively at 32°C after 72 hrs. The highest figure of productivity being 0.031mg $L^{-1}h^{-1}$ was observed after 33 hrs at 32°C. It could also be stated that increasing the temperature from 30°C to 32°C led to significant increase ($p \leq 0.001$) the carotenoids concentration, content and productivity being 55%, 44% and 53% by *R. glutinis* 32 respectively after 72 hrs on modified med -4.

Mahattanatavee & Kulprecha (1991) obtained the highest yield of β-carotene by yeast strains in productive media incubated at 25-3°C. Maximum production of carotenoid pigments was produced by *R. glutinis, L. helveticus* **(Frengova *et al* 1995)** and *R. glutinis, D. castelii* Co-cultures **(Buzzini, 2001)** after 120 hrs at 30°C. The growth of *R. glutinis* 32 and carotenoids production as influenced by different agitation speeds were recorded in **Table (2).** The data indicated that increasing the agitation speed resulted in significant increase ($p \leq 0.001$) the cell dry weight and carotenoids concentration till reaching the maximum (2.8 g L^{-1} & 4.38 mgL^{-1}) at 550 rpm after 72 hrs incubation period.

Table 2. Carotenoids production by *Rhodotorula glutinis* 32 on modified med.4 as affected by different agitation speeds during 96 hours incubation period at 32°C using bioreactor as a batch culture.

Agitation speed	150 rpm			350 rpm (control)			550 rpm			650 rpm			750 rpm		
	Cell	Carotenoid		Cell	Carotenoid		Cell	Carotenoid		Cell	Carotenoid		Cell	Carotenoid	
Time (hours)	dry weight (gl^{-1})	Cons. (mgl^{-1})	$Y_{C/x}$ (mgg^{-1})	dry weight (gl^{-1})	Cons. (mgl^{-1})	$Y_{C/x}$ (mgg^{-1})	dry weight (gl^{-1})	Cons. (mgl^{-1})	$Y_{C/x}$ (mgg^{-1})	dry weight (gl^{-1})	Cons. (mgl^{-1})	$Y_{C/x}$ (mgg^{-1})	dry weight (gl^{-1})	Cons. (mgl^{-1})	$Y_{C/x}$ (mgg^{-1})
0	0.18^{b}	-	-	0.19^{b}	-	-	0.20^{a}	-	-	0.20^{a}	-	-	0.18^{b}	-	-
3	0.21^{d}	0.015^{b}	0.071^{e}	0.24^{c}	0.090^{b}	0.375^{a}	0.23^{c}	0.08^{b}	0.348^{b}	0.33^{a}	0.060^{a}	0.182^{c}	0.31^{b}	0.025^{b}	0.081^{d}
6	0.23^{e}	0.057^{a}	0.248^{a}	0.54^{c}	0.117^{b}	0.217^{b}	0.76^{b}	0.13^{a}	0.168^{c}	0.85^{a}	0.085^{c}	0.100^{d}	0.51^{d}	0.041^{e}	0.080^{e}
9	0.25^{e}	0.062^{d}	0.248^{c}	1.02^{c}	0.340^{b}	0.333^{b}	1.15^{b}	0.58^{a}	0.504^{a}	1.20^{a}	0.102^{c}	0.085^{d}	0.80^{a}	0.059^{d}	0.074^{e}
12	0.33^{e}	0.087^{d}	0.264^{c}	1.23^{c}	0.590^{b}	0.480^{a}	2.50^{a}	0.97^{a}	0.388^{b}	1.35^{b}	0.132^{c}	0.098^{d}	0.93^{d}	0.090^{d}	0.097^{d}
24	0.35^{d}	0.185^{d}	0.529^{a}	1.39^{b}	0.680^{b}	0.489^{b}	2.65^{a}	1.09^{a}	0.409^{c}	1.40^{c}	0.170^{d}	0.121^{d}	1.00^{c}	0.116^{e}	0.116^{c}
27	0.43^{e}	0.225^{c}	0.523^{c}	1.64^{b}	0.985^{b}	0.601^{b}	2.70^{a}	2.02^{a}	0.748^{a}	1.60^{a}	0.284^{c}	0.178^{d}	1.35^{d}	0.162^{e}	0.120^{e}
30	0.45^{e}	0.277^{d}	0.616^{c}	1.85^{a}	1.194^{b}	0.645^{b}	2.70^{a}	2.49^{a}	0.922^{a}	1.65^{a}	0.402^{c}	0.244^{b}	1.40^{d}	0.248^{e}	0.178^{e}
33	0.53^{e}	0.367^{e}	0.692^{b}	2.01^{b}	1.362^{b}	0.678^{c}	2.70^{a}	3.14^{a}	1.163^{a}	1.70^{a}	0.970^{c}	0.571^{d}	1.40^{d}	0.621^{d}	0.444^{e}
48	0.75^{e}	0.397^{e}	0.529^{d}	2.10^{b}	1.44^{c}	0.686^{C}	2.70^{a}	3.96^{a}	1.467^{a}	1.90^{a}	1.523^{b}	0.802^{b}	1.70^{d}	0.842^{d}	0.495^{e}
72	0.80^{e}	0.557^{e}	0.696^{c}	2.40^{d}	1.65^{c}	0.688^{d}	2.80^{a}	4.38^{a}	1.564^{a}	2.60^{a}	2.097^{b}	0.807^{b}	2.50^{c}	1.265^{d}	0.506^{e}
96	0.80^{d}	0.164^{e}	0.205^{e}	2.44^{c}	1.54^{c}	0.631^{b}	2.80^{a}	3.36^{a}	1.203^{a}	2.65^{a}	1.592^{b}	0.601^{c}	2.45^{c}	0.623^{d}	0.254^{a}

Means in the same row within each classification having the different letters are significantly ($p \leq 0.001$) different.

The corresponding figures of carotenoids yield coefficient ($Y_{C/X}$), content and productivity were 1.564 mg g^{-1} cell dry weight, 0.156% and 0.061 mg $L^{-1}h^{-1}$ respectively **(Table 2 & Fig. 2).** Using agitation speed at 550 rpm increased carotenoids concentration, content and productivity approximately 2.65, 2.27 and 2.65 fold, respectively than that produced on culture agitated at 350 rpm.

These results are in partial agreement with those obtained by **Martelli & Da Silva (1993) and Bhosale & Gadre (2001)** who used 400 rpm for optimum carotenoids formation in *R. glutinis* cells.

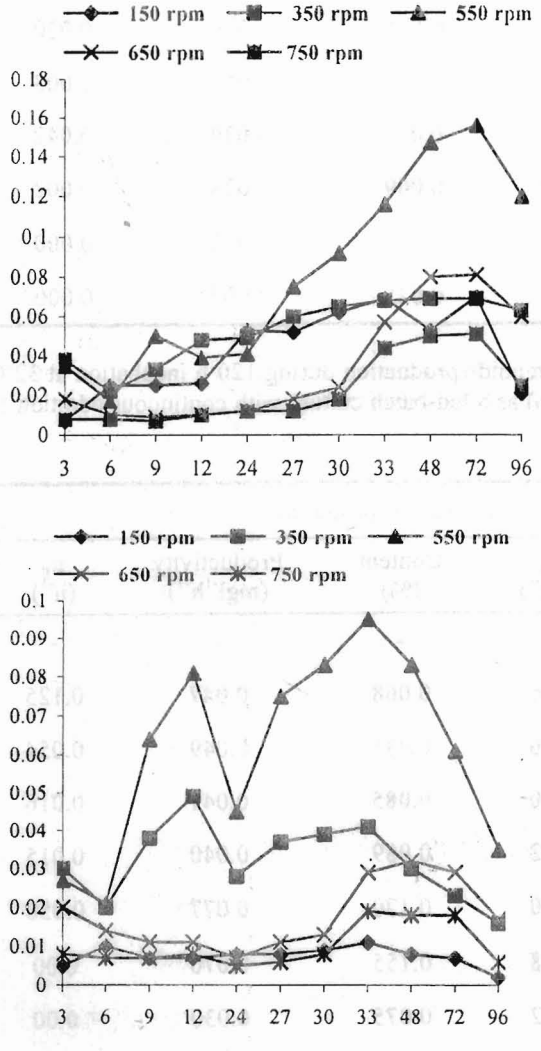

Fig. 2. Parameter of carotenoid produced by *Rhodotorula* glutinis 32 grown on modified med.4 at different agitation speeds for 96 hrs incubation period using bioreactor as a batch culture.

I.2. As two stage batch culture

Data presented in **Table (3)** clearly show that carotenoids concentration, productivity and specific production rate were decreased about 44%, 27% and 50% on molasses medium than that obtained on modified medium 4 (MM-4) after 72 hrs at 32°C. This may be due to the presence of some inhibitory substance such as heavy metals in molasses.

Table 3. Carotenoids production by *Rhodotorula glutinis* 32 on molasses medium during 120 hours incubation period at 32°C using bioreactor (550 rpm) as a two stage batch culture.

Time in hours	Carotenoids		Specific production rate μ_p (h^{-1})
	Concentration (mgl^{-1})	Productivity ($mgl^{-1}h^{-1}$)	
0	0.068	0.003	..
24	0.883	0.034	0.107
48	1.671	0.033	0.027
72	2.365	0.032	0.014
96	2.337	0.024	0.00
120	2.01	0.016	0.00

I.3. Fed batch culture

In fermentation processes where cell growth and/or product formation is inhibited by high substrate concentration or by the accumulation of a byproduct, substrate is intermittently fed to the culture system in order to maintain the substrate concentration below a certain level for enhancement of biological and metabolic activity. So, *R. glutinis* 32 was grown in bioreactor as a fed-batch culture using continuous feeding of glycerol during the first 10 and 30 hrs of fermentation at a rate of 4 gL^{-1} h^{-1} & 1.33 $gL^{-1}h^{-1}$ respectively. Data given in **Tables (4 & 5)** clearly show that the tested strain grow exponentially during the first 24 hrs of fermentation and gave the highest growth after 96 hrs being 3.85 & 3.53 gL^{-1} at 4 $gL^{-1}h^{-1}$ and 1.33 $gL^{-1}h^{-1}$ feeding rate, respectively. At the former feeding rate, the carotenoids production increased gradually during fermentation period, till reach the maximum after 72 hrs, recorded 5.526 mg L^{-1}, 1.7 mg g^{-1}, 0.17% and 0.077 $mgL^{-1}h^{-1}$ for

Table 4. Growth of *Rhodotorula glutinis* 32 and carotenoids production during 120 h incubation at 32°C on modified med.4 using bioreactor (550 rpm) as a fed-batch culture with continuous addition of glycerol at a rate of 1.33 gh^{-1}

Time in hours	Cell dry weight (gl^{-1})	Carotenoids produced				
		Concentration (mgl^{-1})	$Y_{c/x}$ (mgg^{-1})	Content (%)	Productivity ($mgl^{-1}h^{-1}$)	μ_p (h^{-1})
0	0.23	0.00	-	-	-	-
24	1.03	0.532	0.517	0.052	0.022	-
30	1.32	0.684	0.518	0.052	0.023	0.042
48	2.11	0.983	0.466	0.046	0.020	0.020
54	2.30	1.002	0.436	0.044	0.019	0.003
72	3.02	2.134	0.707	0.071	0.030	0.042
78	3.14	2.165	0.689	0.069	0.028	0.002
96	3.53	1.132	0.321	0.032	0.012	0.000
120	3.45	0.876	0.254	0.025	0.007	0.000

Table 5. Growth of *Rhodotorula glutinis* 32 and carotenoids production during 120 h incubation at 32°C on modified med.4 using bioreactor (550 rpm) as a fed-batch culture with continuous addition of glycerol at a rate of 4 gh^{-1}.

Time in hours	Cell dry weight (gl^{-1})	Carotenoids produced				
		Concentration (mgl^{-1})	$Y_{c/x}$ (mgg^{-1})	Content (%)	Productivity ($mgl^{-1}h^{-1}$)	μ_p (h^{-1})
0	0.18	0.00	-	-	-	-
24	1.65	1.116	0.676	0.068	0.047	0.125
30	1.90	1.539	0.810	0.081	0.049	0.054
48	2.40	2.040	0.850	0.085	0.041	0.016
54	2.50	2.231	0.892	0.089	0.040	0.015
72	3.25	5.526	1.700	0.170	0.077	0.050
78	3.55	5.494	1.548	0.155	0.070	0.00
96	3.85	2.895	0.752	0.075	0.030	0.00
120	3.80	2.010	0.529	0.053	0.016	0.00

carotenoids concentration, yield coefficient relative to biomass, content and productivity, respectively. So, this technique increased the concentration, content and productivity to 1.26, 1.09 and 1.26 fold, respectively as compared with that obtained in batch bioreactor technique.

II. Effect of plant or microbial carotenoids administration

II.1. Thyroid hormones (T3 & T4) and daily gain of albino rats

Means of Triiodothyronine (T_3) and Tetraiodothyronine (T_4) levels (µg/dl) of albino rats were illustrated in **Fig. (3)** for control (G.1), administrated plant carotenoids group (G2) and administrated microbial carotenoids group (G3) for 28 days (acute toxicity studies). The statistical analysis showed that the differences in T_3 & T_4 levels among the three groups were insignificant and still within the normal range. **Fujimoto *et al* (1999)** found that the serum triiodothyronine (T_3) and tetraiodothyronine or thyroxine (T_4) concentrations were 6.0 and 40.0 µg/dl, respectively in rats. **Biebinger *et al* (2007)** noticed that repletion of vitamin A in rats with concurrent vitamin A and iodine deficiency had no discernible effects on circulating thyroid hormones.

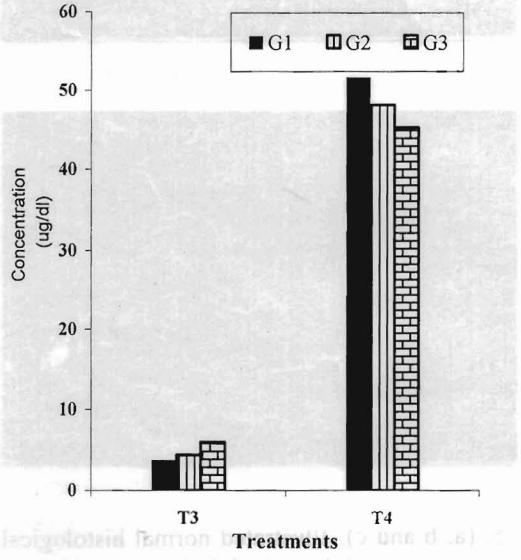

Fig. 3. Serum levels of T3 and T4 in control and treated albino rats administrated with plant or microbial carotenoids for 28 days (acute toxicity studies).

Fig. (4) show that rats which administrated with plant β-carotene (G2) has the highest daily gain (g) followed by rats administrated microbial carotenoids (G3) then the control group (G1), however, the difference of daily gain among the three groups were insignificant. These results may be due to carotenoids which play a role as a precursor of vitamin A. Also, **Simpson (1983)** who reported that carotenoids such as β-carotene is a pro-vitamin A and is necessary for growth and life.

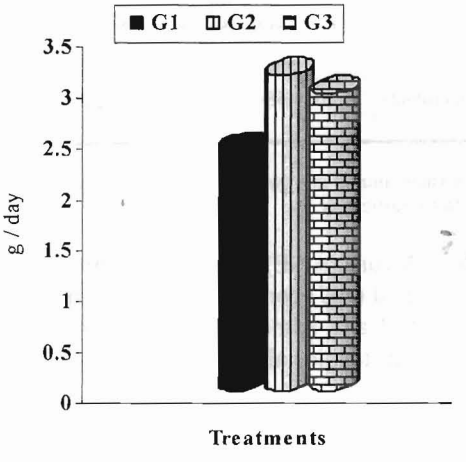

Fig. 4. Daily gain (g) of albino rats, untreated (control) and administrated with plant or microbial carotenoids for 28 days(acute toxicity studies).

II. 2. Liver and kidney functions of albino rats

Overall mean GPT, GOT and ALP concentration which express liver function, and the urea, creatinine and uric acid mean concentrations which express a kidney function of the three groups of albino rats were shown in **Tables (6 and 7).** Analysis of variance revealed that there were no significant differences among groups for the abovementioned parameters except for GPT and uric acid, whereas significant differences were recorded between G3 and the other two groups. These findings were in agreement with those obtained by **Naidu *et al* (1999)** who reported that there were no significant differences in the activity of serum enzymes like alkaline phosphates, ALT (GOT) and AST (GPT) among control and treated groups fed orally *R. gracilis* at 0.5-6.0 g/kg body weight for 14 weeks.

Table 6. Means (± SE) of GPT, GOT and ALP concentrations (U/L) and daily gain (g) for control and administered plant or microbial carotenoids albino rats.

Treatments	GPT (U/L)	GOT (U/L)	ALP (U/L)
Overall mean	26.37±3.652	54.57±5.326	257.29±18.59
G1 (control)	22.50±5.125[b]	39.25±8.790[b]	266.50±37.64[a]
G2 (plant)	17.00±4.581[b]	54.00±7.862[a]	256.80±33.67[a]
G3 (microbial)	38.00±4.584[a]	67.40±7.862[a]	250.4±33.671[a]

Within each column any two means having the same letter do not differ significantly.

Table 7. Means (± SE) of urea, creatinine and uric acid (AU) concentrations (mg/dl) for control and administered plant or microbial carotenoids albino rats.

Treatments	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Overall mean	39.75±3.199	0.788±0.0395	5.78±0.794
G1 (control)	36.14±5.82[a]	0.663±0.0572[a]	4.69±0.957[b]
G2 (plant)	35.75±5.21[a]	0.766±0.0512[ab]	3.61±0.856[b]
G3 (microbial)	46.65±5.21[a]	0.910±0.0512[b]	8.82±0.856[a]

Within each column any two means having the same letter do not differ significantly.

III. Histopathological studies

The present study clearly showed that carotenoids of either plant or microbial origin had no clinical toxicity or carcinogenic signs on liver and kidney tissues of the treated rats during the entire period of treatment (acute toxicity) as illustrated in **Figs. (5 and 6).** Parallel results and conclusion were obtained by **Naidu *et al* (1999)** who reported that feeding high and low concentration of *R. gracilis* (freeze-dried cells) which produced carotenoid pigments to both sues of albino rats for 3 or 14 weeks had no clinical signs of toxicity or mortality of the animals during the entire period of treatment. Moreover, they concluded that *A. gracilis* provided a good source of lipids, carbohydrates and carotenoids. Also, the toxicological studies on rats for 14 weeks did not show any adverse effects.

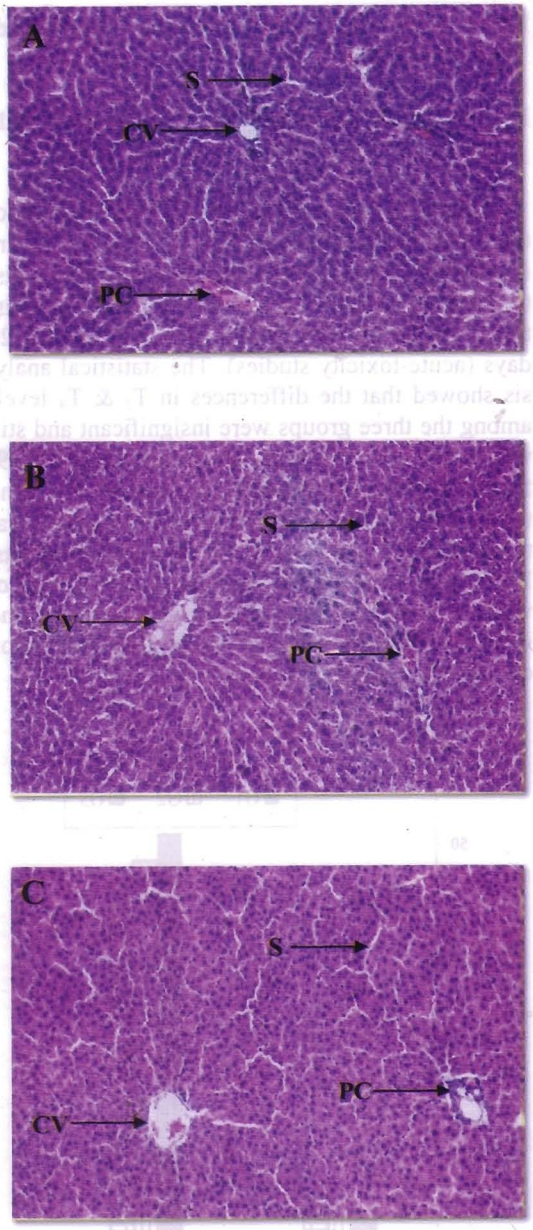

Fig 5. (a, b and c): Illustrated normal histological structure of hepatic lobule , central vein (CV), sinusoids (s) with defuse kupffer cells proliferations and portal canal (pc) of liver of control and administrated plant or microbial β-carotene rats.

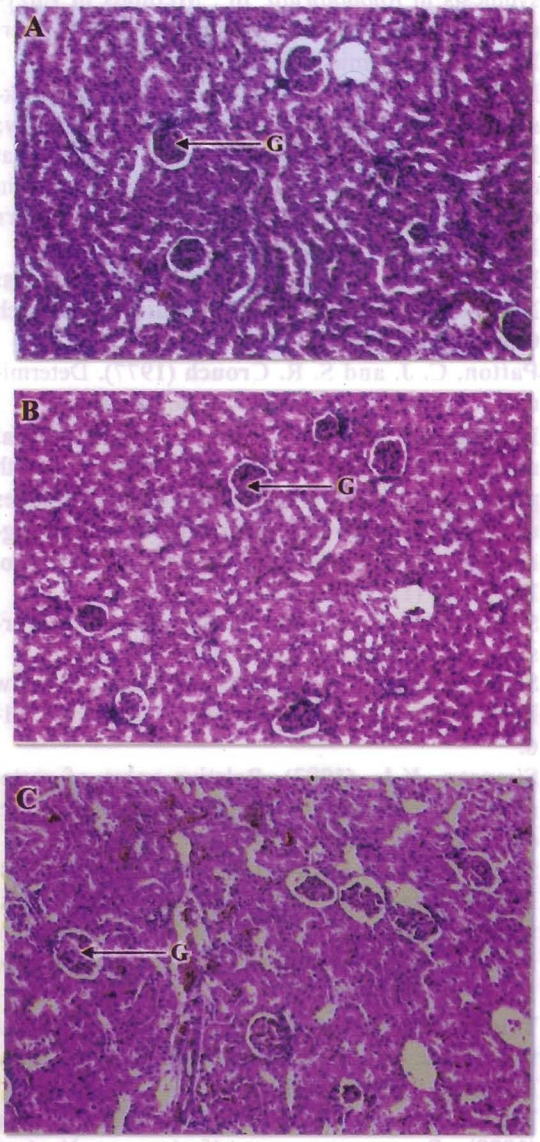

Fig. 6 (a, b and c): Showed normal histological structure of kidney cortex with hyperemic glomeruli (G) and inter lobular blood vessels of kidney of control and administrated plant or microbial B-carotene rats.

From the previous results concerning kidney functions (urea, creatinine and uric acid), liver functions (GOT, GPT and ALP) as well as histopathological studies. It could be recommended to substitute plant carotenoids by microbial one for animal feeding, natural colorant for food industry as a precursor of vitamin A in foods as treatment of photosensitivity diseases. From the economic view, using microbial carotenoids instead of plant carotenoids will save a lot of money and time. This recommendation rate is in line with those obtained by **Tsubokura *et al* (1999)** who stated that the pigments may be used as additives in the food, foodstuff, pharmaceutical or cosmetic industries and also used either as an antioxidant, retinal precursor or as a yellow color. Also, **Lee-Christine *et al* (1999)** suggested that dietary intake of carotenoids influences the risk for certain types of cancer, cardiovascular diseases and other chronic diseases.

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"Production of microbial carotenoids from Agro-raw materials by mixed culture technique"

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حوليات العلوم الزراعية
جامعة عين شمس، القاهرة
مجلد(٥٢)، عدد (١)، ٦١-٧٣، ٢٠٠٧

تقييم الكاروتينات المنتجة بواسطة رودوتوريولا جلوتينس بإستخدام المخمر بطرق تغذية مختلفة على الفئران البيضاء

[٤]

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تم إنتاج الكاروتينات بزراعة *R. glutinis* 32 فى البيئة رقم ٤ المعدلة بإستخدام المخمر كمزرعة ذات دفعة واحدة وذات مرحلتين وذات الدفعة الواحدة المغذاة. إختبرت خمس درجات لكل من حرارة التحضين وسرعات الرج وثبت أن أمثل درجة عند ٣٢° وسرعة ٥٥٠ لفة/ ق. وفى المزرعة ذات المرحلتين إنخفض تركيز الكاروتينات، والانتاجية فى بيئة المولاس بمقدار ٤٤%، ٢٧ % على التوالى بمقارنتها بالبيئة الإنتاجية (المعدلة رقم ٤). تم إختبار معدلين للتغذية ٤، ١,٣٣ جرام جليسرول/ لتر/ ساعة على دفعات لإنتاج الكاروتينات. أدى استخدام ٤ جم جلسرول/ لتر/ ساعة إلى زيادة تركيز الكاروتينات ومحتوى الخلايا منها وإنتاجيتها إلى ١,٢٦ ، ١,٠٩، ١,٢٦ ضعف على التوالى وذلك عند مقارنتها بتلك المنتجة بطريقة المزرعة ذات الدفعة الواحدة بالمخمر. وبتقييم الكاروتين الميكروبى ومقارنته بالكاروتين النباتى وذلك بتقدير تأثرها على كل من هرمونات الدرقية ووظائف الكبد والكلى فى الفئران البيضاء. ثبت من التحليل الإحصائى عدم وجود فروق معنوية بالنسبة لهرمونات T_3, T_4، معدل الزيادة اليومية، انزيمات ALP, GOT بينما ثبت وجود فرق معنوى لانزيم GPT ، تركيزات الكرياتينين وحمض اليوريك فى الدم.

وبالفحص الهستولوجى لكل من أنسجة الكلى والكبد ثبت عدم وجود تغيير فى الأنسجة المختبرة لكل منهما على التوالى.

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