Antioxidant and Antibacterial Effects of Carotenoids Extracted from *Rhodotorula glutinis* Strains

TURKAN MUTLU KECELI*, ZERRIN ERGINKAYA, ESRA TURKKAN and UMIT KAYA

Department of Food Engineering, Faculty of Agriculture, University of Cukurova, TR-01330 Balcali-Adana, Turkey

*Corresponding author: Fax: +90 322 3386614; Tel: +90 322 3387043; E-mail: tkeceli@cukurova.edu.tr

(Received: 9 July 2011;

Accepted: 9 July 2012)

AJC-11817

In this study, carotenoids extracted from the yeast twenty different *Rhodotorula glutinis* strains (M1-M47) isolated from soil, plant, pine and tree leaves, grape, aubergine, pee, parsley and carrot were studied for their antibacterial and antioxidant activity. Carotenoid contents of *Rhodotorula glutinis* strains changed between 0.23 to 1.23 mg L⁻¹. Most of the carotenoids extracted from *Rhodotorula glutinis* strains (sixteen out of twenty) showed both antioxidant and antibacterial effects. Carotenoids extracted from *Rhodotorula glutinis* strains No. 2, 25, 26 did not show any antimicrobial activity and strains No. 1, 33 and 41 did not show any antioxidant effect. Strain No. M38 (0.74 mg L⁻¹) was not effective neither as antibacterial nor antioxidant. The results strongly suggested that the potential utility of carotenoid synthesizing yeast *Rhodotorula* spp as an alternative source of natural preservatives (antioxidant and antimicrobial), carotenoid pigments (colourants) and nutraceuticals.

Key Words: Carotenoid, Antibacterial, Antioxidant, *Rhodotorula glutinis*, Yeast.

INTRODUCTION

There has been much interest in the development of new natural colourants for use in the food industry and food containing natural pigments, which is apparently due to strong consumer demand for more natural products¹. Among pigments with natural origin, carotenoids represent a group of valuable molecules for the pharmaceutical, chemical, food and feed industries not only because they can act as vitamin A precursors, but also for their colouring, antioxidant and possible tumor-inhibiting activity through the removal of oxygen radicals^{2,3}. Carotenoids extracted from different sources have been used as a food supplement or nutritional replenishment for modifying the colour of fats, oils, butter, margarine, cheese, drinks and juices⁴⁻⁶. β -Carotene is used as food colourant in concentrations between 2 and 50 ppm, so that its contribution for the colour of food ranges from yellow to orange⁷. Carotenoids are liposoluble tetraterpenes, usually red or yellow and are one of the most important families of natural pigments widely existing in nature, with over 600 known carotenoids being synthesized from higher plants and microscopic organisms^{5,6}. These pigments have several conjugated double bonds that act as chromophores and thus absorb light in the visible region, which gives them their strong colouration properties⁵. Several microorganisms including bacteria, algae, molds and yeasts are able to produce carotenoids naturally^{2,3}. Yeasts are more convenient than algae or molds for large-scale production in

fermenters, due to their unicellular nature and high growth rate and a greater cell rate^{2,6}. Compared with the extraction from vegetables or chemical synthesis, the microbial production of carotenoids is of paramount interest, mainly because of the problems of seasonal and geographic variability in the production and marketing of various colourants of plant origin and because of the economic advantages of microbial processes using natural low-cost substrates as carbohydrate sources^{3,4,8,9}.

Carotenoids are roughly classified into two groups. One is the hydrocarbon carotenes such as β -carotene, torulene and the other is the oxygenated xanthophylls such as torularhodin and astaxanthin⁵. Various yeasts, in particular, *Rhodotorula*, *Cryptococcus*, *Phaffia* and *Sporobolomyces*, *Rhodospiridium* and *Sporidiobolus* sp. produce a variety of carotenoids that have a broad region of light absorption of 450-550 nm so that the culture broth has a coloured appearance. The fermentation conditions, such as cultivation temperature, lightening, induced substances and inhibitors play important roles in the carotenoid-forming activity of yeasts as well as composition ratio of carotenoids (carotenogenic ratio)^{2,6,10-13}. *Rhodotorula glutinis* is an asporogenous, non-fermenting yeast with carotenoid pigment and is glistening, mucoid, deep coral to salmon pink in colour on most mycological media and therefore of commercial interest^{1,14}. Yeast *Rhodotorula* spp. producing β -carotene, torulene and torularhodin as the major carotenoids pigments) is described as a potential source of

carotenoids with medical or industrial interest^{2,6}. The value of orange-red carotenoids, like torularhodin and torulene for which, at present, no cheap commercially exploitable plant sources are known. The high cost involved in the practical implementation of modern technologies is the major limiting factor for scale-up of fermentation processes for carotenoid production^{2,13}. Carotenoids can be produced in abundance by yeasts, with typical concentrations ranging from 50 to 350 $\mu\text{g g}^{-1}$ dry weight *e.g.* in wild-type strains of *Rhodotorula* spp.¹⁴. The most widely studied health benefits of carotenoids are related to their antioxidative potential, enhancement of immune system function, protection from sunburn and inhibition of the development of certain types of cancers due to quenching singlet oxygen in humans and animals^{5,14,15}. The lack of dietary carotenoids may result in xerophthalmia, keratomalacia, blindness and in many cases, death¹⁵. Carotenoids act as chain breaking antioxidants by scavenging and deactivating free radicals both *in vitro* and *in vivo*¹⁶⁻²⁰. Free radical scavenging and antioxidant activities of metabolites produced by carotenogenic yeasts of *Phaffia rhodozyma*, *Rhodotorula* spp. and *Sporobolomyces* spp. were studied by several authors¹⁹⁻²³. An²⁰ has showed that some strains of *Phaffia rhodozyma* produces carotenoids which are antioxidants and protect cells against harmful oxygen radicals. Free radical scavenging and antioxidant activities of metabolites produced by carotenogenic yeasts of *Rhodotorula* spp. and *Sporobolomyces* spp. were studied by Rapta *et al.*²². They found that the antioxidants present in yeast's walls showed higher ability to scavenge free radicals than those from inside cells.

There is no study on both antioxidant/antimicrobial activity of *Rhodotorula glutinis* strains which has been known to be good producer of carotenoids. Due to increasing demand of natural food and alternative for synthetic food colourants carotenoids from *Rhodotorula* may be used as both natural antioxidants and antimicrobial agents in food products. Therefore, the aim of present investigation was to study antioxidant and antibacterial effects of carotenoid extracts obtained from *Rhodotorula glutinis* strains.

EXPERIMENTAL

Rhodotorula glutinis and bacteria strains were obtained from the collection of University of Cukurova and Ankara, Turkey and University of Hohenheim, Germany, respectively. AAPH (2,2'-azobis amidino propane dihydrochloride), Nutrient Broth, Yeast Extract Broth, Malt Extract Broth, Agar Agar, NaCl, Trypton or peptone were obtained from Aldrich, Merck and Oxoid, Turkey. Refined sunflower oil was purchased at a retail outlet. Other chemicals and reagents for the experiments were of analytical grade and purchased from Sigma-Aldrich, Turkey.

General procedure:

Test bacteria strains and growth conditions: The following strains of pathogen bacteria were used: *Staphylococcus aureus* (B50), *Bacillus subtilis* (B40), *Bacillus cereus* (B41), *Salmonella Enteritidis* (B42) and *Escherichia coli* (B43). Bacterial strains were inoculated into nutrient broth and incubated at $37 \pm 1^\circ\text{C}$ for 24 h and then wet cells were collected by centrifuging the growth medium at 5000 rpm for

10 min. The bacterial concentration was determined by measuring the optical density at 578 nm with UV-VIS spectrophotometer (Lambda Bio 20, Perkin-Elmer) and was adjusted to 10^3 to 10^6 cfu/mL.

Carotenoid production: *Rhodotorula glutinis* spp. strains (M1 to M47) were transferred into YME agar and incubated at $25 \pm 1^\circ\text{C}$ for 72 h and then yeast strains were inoculated into 5 mL YME agar and incubated at $25 \pm 1^\circ\text{C}$ for 24 h. Later incubation of 0.1 mL yeast strains were inoculated into 5 mL YME broth and incubated at $25 \pm 1^\circ\text{C}$ for 10 days. The yeast identification key given by Barnet *et al.*²⁴ and API 20 C AUX (Biomerieux) identification kits were used to identify *Rhodotorula glutinis* spp. strains. At the end of the incubation period the culture media were centrifuged (5000 rpm, 10 min) and the pellets were taken for the extraction of carotenoids. All the experiments were done triplicate.

Cell rupture and carotenoid extraction: Yeast cells were harvested from the growing cultures by centrifugation (5000 rpm, 10 min), washed by water and washed twice with 9 % NaCl solution. Then, carotenoids were extracted by adding 1 mL dimethyl sulfoxide, acetone and petroleum ether, respectively to the yeast pellet by vortexing. Upon standing for 10-15 min, the tubes were centrifuged as before. The supernatants were then decanted into small separatory funnels. Upon addition of 1 mL of 20 % NaCl solution, a clear-cut separation of the two phases occurred and if carotenoid pigments were present, the upper (epiphasic) petroleum ether layer was coloured yellow, orange, pink or intense red. The absorbance spectra at 485 nm were recorded on a UV-VIS spectrometer (Lambda Bio 20, Perkin-Elmer) spectrophotometer. The total carotenoid composition was calculated by using formula as mg of equivalent torulene/L of culture fluid^{14,10-12}:

$$\text{Total carotenoid (mg/L)} = (V_{\text{PET}} \times A_{485} \times D) / E_1^{\%1}$$

where, V_{PET} : volume of petroleum ether (mL), A_{485} : absorbance at 485 nm, D: Dilution rate, $E_1^{\%1}$: absorption coefficient for torulene.

Preparation of emulsions with carotenoid extracts and oxidation: Each sunflower oil-in-water emulsion (10 g) was prepared by cooling a solution of distilled water (9 g) containing Tween 20 (0.1 g) as emulsifier and 0.1 mL of AAPH as a chemical initiator. AVC-50 sonicator was immersed in the solution (placed in an ice bath) and 1 g of purified sunflower oil (containing carotenoids at the required concentration level of 0.2-1 g/L obtained from *Rhodotorula glutinis* spp. strains (M1-M47) was added dropwise^{16,17}. After their preparation, emulsion samples were transferred to screw-capped sample vials and held in a shaking water bath where allowed to oxidize at 60°C for 3 h. This procedure was accomplished two times for each carotenoid extracts obtained from *Rhodotorula glutinis* spp. strains (M1 to M47).

Detection method

Determination of antioxidant activity: The oxidative stability of the emulsions (containing carotenoid extracts obtained from *Rhodotorula glutinis* spp. strains) was determined by monitoring the formation of conjugated dienes during oxidation at 60°C for 3 h. Emulsion samples (0.1 mL) were periodically removed during the period of oxidation, diluted to 10 mL in

ethanol and their absorbance at 233 nm was determined with a UV-VIS spectrometer (Lambda Bio 20, Perkin-Elmer) as an oxidative indicator as described by the AOCS official method Ti La-64²⁵. Analysis was done in duplicate.

Determination of antibacterial activity: Nutrient agar medium (g/L: peptone 5.0; beef extract 1.5; yeast extract 1.5; NaCl 5.0; agar 20; pH 7.5) was prepared and autoclaved at 121 °C for 20 min. Sterilized petri plates were prepared with an equal thickness of nutrient agar. Test organisms were grown overnight at 37 °C, 120 rpm in 10 mL nutrient broth. This broth was used for seeding the agar plates. One millilitre of each pathogen bacteria was spread over the surface of nutrient agar plate with a bent glass rod and 200 µL of carotenoid extraction were added to the wells of these plates. After incubation at 37 ± 1 °C for 48-72 h the potency of carotenoid extracts was determined by measuring the diameter of the inhibition zones. This procedure was accomplished two times for each carotenoid extracts obtained from *Rhodotorula glutinis* spp. strains (M1 to M47)^{23,26}.

Statistical analysis: The results were shown as mean values. The effect of carotenoid extracts from *Rhodotorula glutinis* spp. was evaluated by analysis of variance (ANOVA). According to the results of ANOVA test Duncan's multiple range test was used to determine the significance at $p < 0.05$ levels²⁷.

RESULTS AND DISCUSSION

The isolation source and carotenoid amount of the extracts obtained from *Rhodotorula glutinis* strains (M1 to M47) are presented at Table-1. *Rhodotorula* strains are isolated from soil (8), plant (2), pine and tree leaves (3), grape (2), aubergine (2), pee (1), parsley (1) and carrot (1) respectively (Table-1). Yeasts are important members in many ecosystems and also form a major component of the population²⁸.

The carotenoid amount of the *Rhodotorula glutinis* strains changed between 0.23 to 1.23 mg/L and the highest was present in strain no. M28 (isolated from aubergine) and the lowest was present in strain no. M21 (isolated from soil). It was reported that carotenoid production of *Rhodotorula glutinis* was 19.4 mg/L and the 81 % of it was formed by β -carotene⁷. However, it was also found that 330 µg per g of dry biomass carotenoid production can be achieved in 6 or 7 days of fermentation by the cultivation of some *Rhodotorula* spp as well as *Sporobolomyces* spp used for the carotenoid production⁷. Squina *et al.*²⁹ found that carotenoid production of *R. glutinis* was 197.2 µg/g dry mass after 96 h incubation time. The total carotenoid production of *Rhodotorula* spp. was reported to be ranging from 70 to 1700 µg/g dry mass in the literature²⁸. Buzzini and Martini³⁰ found that the total carotenoid content of *Rhodotorula glutinis* were changed between 1.8 and 5.95 mg /L after 120 h and changed depending on carbohydrate source and fermentation time. Total carotenoid content of *Rhodotorula glutinis* DBVPG 3853 were 0.55 mg/L after 24 h incubation time and increased up to 10 fold about 5.95 mg/L after 120 h incubation time when grape must was used as a carbon source. Our results were lower than these findings. Since fermentation time for *Rhodotorula* strains were 24 h and our results could therefore be lower than their findings. It is obvious that the incubation time and carbon source is quite important for carotenoid production of *Rhodotorula* strains.

TABLE-1
STRAINS SOURCE, CAROTENOID CONCENTRATION AND
ANTIOXIDANT EFFECT OF CAROTENOID EXTRACTS
OBTAINED FROM *Rhodotorula glutinis* STRAINS

Yeast strain number	Source for isolation	Concentration (mg L ⁻¹)	Absorbance (233 nm)
Control	-	0	3.27 ^a
M1	Plant	0.40	2.92 ^{abc}
M2	Plant	0.63	2.80 ^{defg}
M21	Soil	0.23	2.49 ^{defg}
M22	Pine leaves	0.74	2.28 ^{fg}
M24	Grape	0.84	2.33 ^{fg}
M25	Grape	0.49	2.12 ^{ghi}
M26	Soil	0.56	1.89 ^{hij}
M28	Aubergine	1.23	2.60 ^{cdef}
M29	Tree leaves	1.07	2.51 ^{defg}
M30	Pee	0.93	2.39 ^{efg}
M31	Soil	0.60	1.82 ^{hij}
M33	Soil	0.49	3.30 ^a
M35	Parsley	0.94	2.45 ^{defg}
M37	Soil	0.89	1.80 ^{ij}
M38	Aubergine	0.74	2.99 ^{abc}
M39	Tree leaves	0.87	2.45 ^{defg}
M40	Carrot	0.95	1.61 ^j
M41	Soil	0.75	3.06 ^{ab}
M42	Soil	0.98	2.83 ^{bcd}
M47	Soil	0.99	2.21 ^{fgh}

The antioxidant activity of carotenoid extracts obtained from 20 different *Rhodotorula glutinis* strains were determined (Table-1). There were no activity found when the carotenoid concentration was 0.2 or 0.5 g/L (unpublished results). Therefore, the oxidative stability of carotenoid extracts (at 1 g L⁻¹ in oil) from *Rhodotorula glutinis* was determined after 3 h of AAPH-initiated oxidation at 60 °C in oil-in-water emulsions and expressed by absorbance at 233 nm.

The results indicated that some strains containing carotenoid extracts obtained from *Rhodotorula glutinis* were effective as antioxidants ($p < 0.05$). Sixteen strains were active as antioxidant whereas four strains of *Rhodotorula glutinis* showed no antioxidant character. The carotenoid extracts obtained from M26 (0.56 mg L⁻¹) ≤ M31 (0.60 mg L⁻¹) ≤ M37 (0.89 mg L⁻¹) ≤ M40 (0.95 mg L⁻¹) strains of *Rhodotorula glutinis* isolated from carrot and soil respectively, inhibited the AAPH-initiated oxidation and exerted an antioxidant character when compared to the control ($p < 0.05$). Table-1 shows that, the rest of carotenoid extracts obtained *Rhodotorula glutinis* strains isolated from soil, plant, pine and tree leaves, grape, aubergine, pee and parsley, respectively showed slight antioxidant activity ($p \leq 0.05$). However, the carotenoids extracted from M33 (0.49 mg L⁻¹), M41 (0.75 mg L⁻¹), M38 (0.74 mg L⁻¹) and M1 (0.40 mg L⁻¹) strains of *Rhodotorula glutinis* isolated from soil, aubergine and plant respectively, did not inhibit the AAPH-initiated oxidation compared to the control ($p > 0.05$).

According to Table-1, it was found that although carotenoid concentration increased from 0.56 to 0.95 mg L⁻¹ the antioxidant activity still remained unchanged ($p < 0.05$). It is suggested that the antioxidant activity of carotenoid extracts obtained from *Rhodotorula glutinis* were strongly related to the strain and possible isolation source but not depend on carotenoid content. Present results are consistent with the

results previous authors tested carotenoids in oil-in-water emulsions^{14,16,18,19}. The antioxidant potential of certain carotenoids in organic solutions is related to oxygen concentration, the chemical structure of carotenoids¹⁴. However, whether the structure of carotenoids may determine their functionality is a matter of scientific importance, which still has not been elucidated¹⁷. Different carotenoids are derived essentially by modifications in the base structure by cyclization of the end groups and by introduction of oxygen functions giving them their characteristic colours and antioxidant properties. The most important biological function of carotenoids is as antioxidants owing to their potential to inactivate singlet oxygen and to quench carboxy radicals¹. Present results are convincing scientific evidence in support of the association between carotenoids and antioxidant properties.

The antibacterial effect of carotenoid extracts obtained from 20 different *Rhodotorula glutinis* strains were determined on 5 different pathogen bacteria by using agar diffusion method. The results are shown at Table-2.

TABLE-2
ANTIBACTERIAL EFFECT OF CAROTENOID EXTRACTS
FROM *Rhodotorula glutinis* ON PATHOGEN BACTERIA
AS EXPRESSED AS INHIBITION ZONE (mm)

Yeast strain number*	SA	BS	BC	SE	EC
M1 (0.40 mg L ⁻¹)	8.3	-	-	10	-
M2 (0.63 mg L ⁻¹)	-	-	-	-	-
M21 (0.23 mg L ⁻¹)	-	9	10	-	-
M22 (0.74 mg L ⁻¹)	-	-	-	11	-
M24 (0.84 mg L ⁻¹)	17	-	-	12.6	12
M25 (0.49 mg L ⁻¹)	-	-	-	-	-
M26 (0.55 mg L ⁻¹)	-	-	-	-	-
M28 (1.23 mg L ⁻¹)	-	8	8.6	11.3	-
M29 (1.07 mg L ⁻¹)	15.7	9.6	8.3	12.4	12
M30 (0.93 mg L ⁻¹)	16.7	-	-	11.6	11
M31 (0.60 mg L ⁻¹)	6.3	12	10	-	-
M33 (0.49 mg L ⁻¹)	-	9	9.3	6.3	-
M35 (0.94 mg L ⁻¹)	-	12	6.6	13.3	-
M37 (0.89 mg L ⁻¹)	-	9.3	6.6	13.7	-
M38 (0.74 mg L ⁻¹)	-	-	-	-	-
M39 (0.87 mg L ⁻¹)	16.3	-	-	-	11.7
M40 (0.95 mg L ⁻¹)	-	11	9	11.3	9.3
M41 (0.76 mg L ⁻¹)	6.3	11	-	10	-
M42 (0.98 mg L ⁻¹)	13	-	-	11.7	9.6
M47 (0.99 mg L ⁻¹)	-	11.3	8.3	11.7	-

SA = *Staphylococcus aureus* (B50); BS = *Bacillus subtilis* (B40); BC = *Bacillus cereus* (B41); SE = *Salmonella Enteritidis* (B42); EC = *Escherichia coli* (B43); *(-) = No effect

Similar to antioxidant activity, 16 strains obtained from *Rhodotorula glutinis* were active as antibacterial whereas four strains showed no antibacterial effect (Table-2). It was found that carotenoid extract obtained from *Rhodotorula glutinis* strain No. M29 (1.07 mg L⁻¹) isolated from tree leaves is the most effective antibacterial agent against all tested pathogen bacteria. The results showed that carotenoids extracted from some *Rhodotorula glutinis* strains isolated from soil, plant, pine and tree leaves, grape, aubergine, pee and parsley, respectively showed slight antibacterial activity (Table-2). However, carotenoid extracts obtained from strain No. M2 (63 mg L⁻¹), M25 (0.49 mg L⁻¹), M26 (0.56 mg L⁻¹) and M38 (0.74

mg L⁻¹) strains of *Rhodotorula glutinis* isolated from plant, grape, soil and aubergine, respectively did not show any antibacterial activity on 5 pathogen bacteria and M 22 (0.74 mg L⁻¹) isolated from pine leaves did not show antibacterial activity except on *Salmonella Enteritidis* (B42).

The results indicated that in the event of 10³ cfu/mL bacteria concentration; carotenoids extracted from *Rhodotorula glutinis* spp. No. M24, M30, M39, M29, M42, M1 and M41 isolated from grape, tree leaves, plant and soil, respectively were effective as antibacterial on *S. aureus*. The results also showed that carotenoids extracted from *Rhodotorula glutinis* spp strain No. M31, M35, M47, M40, M 41, M37, M21, M33 and M28 isolated from soil, parsley, carrot, aubergine were effective on *B. subtilis*, strain No. M21, M31, M33, M40, M28, M29, M47, M35 and M37 isolated from soil, carrot, aubergine, tree leaves and parsley were effective on *B. cereus*, strain No. M37, M35, M24, M29, M42, M47, M30, M28, M40, M22, M1, M41 and M33 isolated from soil, parsley, grape, tree leaves, pee, aubergine, carrot, pine leaves and plant were effective on *Salmonella Enteritidis*, strain No. M24 M29, M39, M30, M42 and M40 isolated from grape, tree leaves, pee, soil and carrot were effective on *E. coli*, respectively. When bacteria concentration was 106 cfu/mL antibacterial affect was significantly decreased.

The results strongly showed that 13, 10, 9, 8 and 6 strains of *Rhodotorula glutinis* were effective as antibacterial on *Salmonella Enteritidis*, *B. subtilis*, *B. cereus*, *S. aureus* and *E. coli*, respectively. Thus *Salmonella Enteritidis* B42 were found to be the most susceptible and *E. coli* B 43 were found to be the most resistant food pathogen against carotenoid extracts obtained from *Rhodotorula glutinis* spp. Present results are consistent with some previous studies that have shown that species belong to the genera of *Rhodotorula* have been known to form carotenoids with some antibacterial effects^{3,11-13}. Leaf surfaces are colonized by members of several genera of saprophytic yeasts that provide a natural barrier against plant pathogens²⁸. Present results are strongly convincing that carotegenic yeast like *Rhodotorula glutinis* may have a protective role against pathogens as antibacterial agent.

It was found that antibacterial effect is directly associated both with the *Rhodotorula glutinis* strains, source for isolation and the concentrations of the pathogen bacteria but not depend on carotenoid content. It has been thought that carotenoids extracted from some strains of yeast *Rhodotorula glutinis* having antibacterial activities would play an important role in the reduction of food pathogen bacteria including *E. coli* as well as *Salmonella Enteritidis*, *B. subtilis*, *B. cereus* and *S. aureus* and thereby replace the artificial preservatives during production, storage and packaging of foods.

Conclusion

It was concluded that carotenoids extracted from some strains of *Rhodotorula glutinis* (M21, 22, 24, 28, 29, 30, 31, 35, 37, 39, 40, 42 and 47) can effectively be used to obtain both antioxidant and antibacterial effect. These strains can be isolated best from tree leaves, pees, parsley and carrots and these strains produce high quality carotenoids to be used as preservatives. The antioxidant or antibacterial effect of carotenoids is significantly depend on source of isolation and

carotenoid composition rather than carotenoid content. Therefore, the addition of carotenoid extracts obtained from some strains of *Rhodotorula glutinis* in food not only enhances the value addition by making the food more presentable but also shall address the issue of food supplementation with substances that are good antimicrobial and antioxidants. It is obvious that commercial carotenoid biosynthesis using microbial biotechnology and their use seems to have a great and colourful future. There is a need to improve fermentation strategies such that the intracellular accumulation of carotenoid from *Rhodotorula* is feasible on an industrial scale. Manipulation of external and cultural stimulants will allow high carotenoid production from *Rhodotorula* strains to be scaled-up for commercialization². Carotenoids extracted from some strains of *Rhodotorula glutinis* are possible to be used in foods as natural preservatives as well as colourants and recommended as food supplements.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support of University of Cukurova (Project Number: ZF2006BAP9).

REFERENCES

1. R. Siva, M.G. Palackan, L. Maimoon, D. Bhakta, P. Palamurgan and S. Rajanarayanan, *Food Sci Biotech.*, **20**, 7 (2011).
2. G.I. Frengova and D.M. Beshkova, *J. Indian Microbiol Biotechnol.*, **36**, 163 (2009).
3. E.A. Johnson and W.A. Schroeder, *Adv. Biochem. Eng. Biotechnol.*, **53**, 121 (1995).
4. M.M. El-Sheekh, Y.A.G. Mahmoud and A-S. W. Hamza, *Folia Microbiol.*, **55**, 61 (2010).
5. B.K. Kim, P.K. Park, H.J. Chae and E.Y. Kim, *Korean J. Chem. Eng.*, **21**, 689 (2004).
6. D.Y. Chu, Y.Q. Xie, S.Q. Hong, X.Y. Yan, H.W. Tong and S.M. Liu, *Asian J. Chem.*, **22**, 2635 (2010).
7. B.D. Ribeiro, D.W. Barreto and M.A. Z. Coelho, *Food Bioprocess Technol.*, **4**, 693 (2011).
8. A. Das, S.H. Yoon, S.H. Lee, J.Y. Kim, D.K. Oh and S.W. Kim, *Appl. Microbiol. Biotechnol.*, **77**, 505 (2007).
9. J.A. Del Campo, M. Garcia-Gonzalez and M.G. Guerrero, *Appl. Microbiol. Biotechnol.*, **74**, 1163 (2007).
10. A.M. Martin, L. Chun and R.P. Thakor, *J. Ferment. Bioeng.*, **76**, 321 (1993).
11. G. Frengova, E. Simova, K. Pavlova, D. Beshkova and D. Grigorova, *Biotechnol. Bioeng.*, **44**, 888 (1994).
12. M.E. Jaramillo-Flores and R.Mora-Escobedo, *Food Science and Food Biotechnology*, CRC Press, p. 178 (2003).
13. M.A. Borowitzka, *Carotenoid Production Using Microorganisms*, AOCS Press, p. 560 (2005).
14. P. Davoli, V. Mierau and R.W.S. Weber, *Appl. Biochem. Microbiol.*, **40**, 392 (2004).
15. G.K. Chandi, S.P. Singh, B.S. Gill, D.S. Sogi and P. Singh, *Food Sci. Biotechnol.*, **19**, 881 (2010).
16. S. Kiokias and M.H. Gordon, *Food Chem.*, **83**, 523 (2003).
17. S. Kiokias and V. Oreopolou, *Innov. Food Sci. Emerg. Technol.*, **7**, 132 (2006).
18. K. Shindo, K. Kikuta, A. Suzuki, A. Katsuta, H. Kasai, M. Yasumoto-Hirose, Y. Matsuo, N. Misawa and S. Takaichi, *Appl. Microbiol. Biotechnol.*, **74**, 1350 (2007).
19. L. Zhang, Q. Yang, C. Fang, Q. Zhang and Y. Tang, *Arch. Microbiol.*, **188**, 411 (2007).
20. G.H. An, *Appl. Biochem. Biotechnol.*, **66**, 263 (1997).
21. H. Sakaki, H. Nochide, S. Komemushi and W. Miki, *J. Biosci. Bioeng.*, **93**, 338 (2002).
22. P. Rapta, M. Polovka, M. Zalibera, E. Breierova, I. Zitnanova, I. Marova and C. Milan, *Biophys. Chem.*, **116**, 1 (2005).
23. S. Nanosambat and P. Wuttigol, *Food Sci. Biotechnol.*, **20**, 45 (2011).
24. J.A. Barnett, R.W. Payne and D. Yarrow, *Yeasts Characteristics and Identification*, Cambridge University Press, Cambridge, London, New York, p. 887 (1990).
25. D. Firestone, *AOCS Official Methods and Recommended Practices* (1989).
26. R. SübMuth, J. Eberspächer, R. Haag and W. Springer, *Biochemisch Mikrobiologisches, Praktikum*. Thieme Verlag-Stuttgart, p. 409 (1987).
27. R.G.D. Steel and J.H. Torrie, *Principles and Procedures of Statistics*, McGraw Hill Book Co. Inc. New York (1980).
28. E. Slavikova, R. Vadkertiova and D. Vranova, *Ann. Microbiol.*, **59**, 419 (2009).
29. F.M. Squina, F. Yamashita, J.L. Pereira and Z. Mercadante, *Food Biotechnol.*, **16**, 227 (2002).
30. B.P. Buzzini and A. Martini, *Bioresour. Technol.*, **71**, 41 (1999).