International Journal of Fermented Foods: v.3.n.2 p-105-113 Dec. 2014

DOI No. 10.5958/2321-712X.2014.01312.X

Effect of Ph and Temperature on Carotenoid Pigments produced from *Rhodotorula Minuta*

Shivalkar Yadav K[1]* and Prabha R.[2]

[1]*Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Bengaluru -560024, India*
[2]*Asst.Quality Control Officer, APDDCF, Hyderabad-500007, India.*

**Corresponding author : shivalkaryadav@gmail.com*

ABSTRACT

The main criterion for food selection is colour. Colours of the commercial products play a vital role to attract the consumers and also represent the quality of the products. Some synthetic colours are detrimental to human health and hence food industry is looking for natural pigment. Rhodotorula is a carotenoid biosynthetic yeast producing yellow or orange red colonies. The carotenoid pigment extracted from *Rhodotorula minuta* grown in coconut water as natural medium incubated at 30^0C for 3-5 days period showed stability at acidic, neutral and alkaline P^H. Heating the pigment to lab pasteurization (63^0C/30min), boiling (100^0C/10min) and sterilization (121^0C/15min) reduced the intensity of extra and intracellular pigment by 15 to 20 per cent. Out of both intracellular behaved better than extracellular pigment of *Rhodotorula minuta*.

Keywords: Carotenoids, Microbial pigments, Natural food colourants, Stability, Extracellular, Intracellular pigment

Many advances in the developments of food colours have been made over the last 25 years, particularly in terms of harmonized legislation and advances in processing and formulation technology, there is still scope for future developments. The overall forecast for colour market is to grow in line with technological and sociological changes that will lead to an overall increase in processed foodstuffs. It is thought that the natural colour market will grow on a global scale at a greater rate than synthetic colours

owing to a continued consumer pressure to 'go natural'. (Dowman, 2000).

Carotenoids are the most wide spread naturally occurring yellow, orange and red pigments. The abundance of carotenoids in nature is probably due to their relatively simple biosynthetic pathway, which has been demonstrated in higher plants and algae but also in bacteria and yeasts. For microbial carotenoids there are at least three important reasons of analyzing the entire spectrum and the correct amount: firstly, microorganisms offer economical biotechnological production of carotenoids and provide an alternative to chemical synthesis. In bacteria and in yeast, carotenoids have been considered as typical secondary metabolites, playing a certain role in the survival of the fittest microorganisms. Industrially, carotenoid pigments are utilized as food colourants and feed supplements in fish and poultry (Frengova *et al.*, 2003).

Recently carotenoids have attracted greater attention due to their beneficial effect on human health such as involvement in cancer prevention, reduction of the risk for degenerative diseases such as cardiovascular diseases, macular degeneration and cataract and enhancement of immune response. (Costa *et al.*, 2005; Iriani *et al.*, 2005). Thus, a comprehensive screening of the microbial carotenoid spectrum could help to identify novel compounds providing beneficial effects.

Rhodotorula is a carotenoid biosynthetic yeast, part of the Basidiomycota phylum, easily identifiable by distinctive yellow, orange/red colonies (Postgate, 1994). The main carotenoids produced were identified as torularhodin, torulene, γ-carotene, and minute β-carotene in Rhodotorula species.

Latha (2005) found that the *Rhodotorula glutinis* DFR-PDY which was able to grow and form pigments under a wide range of initial pH conditions from 2.5 to 9.5. The cell dry weight increased gradually with an increase in the pH of the modified Czapek dox broth; while the highest carotenoid yield (3.3mg/L) was obtained at pH 5.5 and can be considered as the optimum pH for pigment production.

The specific growth rate of the *Rhodotorula glutinis* increased notably with raise in temperature up to 30°C and lessened sharply above 30°C due to the denaturation of the enzyme system of microorganism at higher temperatures. The highest specific growth rate of yeast was 0.238 h−1 at 30°C (Aksu, 2007). In the present study the extracted pigment from *Rhodotorula minuta* was subjected to pH and temperature stability.

MATERIALS AND METHODS

Cultures and their maintenance

Characterized *Rhodotorula minuta* RAI_3 obtained from air of dairy environment was used in the present study for extraction of the pigment. The yeast cultures were maintained as slant cultures on Malt Yeast Extract Agar (MYEA) and working cultures in MYEB **(**Kaur *et al*.**,** 2009**)** broth with incubation at 30°C/3-5 days.

Production and extraction of pigment

R.minuta RAI_3, inoculated to broth media such as sterile MYEB as semi synthetic medium, coconut water as natural medium and rice as natural solid medium and incubated at 30°C for 3rd, 6th and 9 days respectively (write the reference of your article about to publish) .

pH adjustment and heat treatment of pigment

pH of the acetone extraction of extra and intracellular pigment of Rhodotorula minuta was adjusted to pH 4, 5, 7, 8 and 9 using 0.1N NaOH and HCl. and absorbance was measured at 520nm. Acetone extracted pigment solution of Rhodotorula minuta was heated laboratory pasteurization (63°C for 30 min), boiling (100°C/ 10 min) and sterilization temperature (121°C/ 15 min) and change in absorbance was measured at 520 nm.

RESULTS AND DISCUSSION

Effect of different pH on extra and intracellular pigment of *Rhodotorula minuta* RAI_3 *from sterile MYEB, coconut water and rice*

The pigment solution showed its stability at pH 7 with no change in absorbance (0.135 /ml) and (0.410 /ml) compared to control (pH 6). But at acidic pH 4 and 5 absorbance of pigment decreased as shown in Tables 1 and 2. Maximum decrease in absorbance was observed at pH 4 (0.044 / ml and 0.082 /ml for extra and intracellular respectively). Adjusting the pH of extra and intracellular pigment to pH 8 and 9 found decrease in the absorbance. Statistically significant difference was noticed when acetone extracted pigment was of *Rhodotorula minuta* adjusted to pH 4, 5, 7, 8 and 9. Consistent pH changes were noticed in all the 3 media .Media has no effect on stability on pigment but pH has the significant effect on intensity of pigment. From the observations it can be concluded that the pigment

Table 1. Effect of different pH on extracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, coconut water and rice

Medium used for growth of pigment	Effect of different pH										
	4pH		5pH		6 pH**	7 pH	8 pH		9 pH		CD (P≥ 0.05)
	Extra-cellular (µg/g of dry cell mass)	Percent retention	Extra-cellular (µg/g of dry cell mass)	Percent reten-tion	Extra-cellular (µg/g of dry cell mass)	Extra-cellular (µg/g of dry cell mass)	Extra-cellular (µg/g of dry cell mass)	Percent reten-tion	Extra-cellular (µg/g of dry cell mass)	Percent reten-tion	
MYEB	0.055 (0.071)	43%	0.068 (0.088)	53%	0.128 (0.167)	0.130 (0.169)	0.092 (0.120)	72%	0.054 (0.070)	42%	0.05
Coconut water	0.044 (0.057)	36%	0.058 (0.075)	47%	0.121 (0.158)	0.135 (0.176)	0.084 (0.109)	69%	0.050 (0.065)	41%	
Rice	0.069 (0.090)	46%	0.091 (0.118)	60%	0.151 (0.197)	0.160 (0.209)	0.096 (0.125)	63%	0.052 (0.067)	34%	
CD (P≥ 0.05)	0.09										

Note: Acetone extracted pigment was used.

- pH 6 indicate the control i.e. pH of the extracted pigment
- Values in parenthesis indicate the quantity of pigment

Table 2. Effect of different pH on intracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB coconut water and rice

Medium used for growth of pigment	Effect of different pH										
	4pH		5pH		6 pH**	7 pH	8 pH		9 pH		CD (P≥ 0.05)
	Intra-cellular (µg/g of dry cell mass)	Percent reten-tion	Intra-cellular (µg/g of dry cell mass)	Percent retention	Intra-cellular (µg/g of dry cell mass)	Intra-cellular (µg/g of dry cell mass)	Intra-cellular (µg/g of dry cell mass)	Percent reten-tion	Intra-cellular (µg/g of dry cell mass)	Percent reten-tion	
MYEB	0.099 (0.129)	36%	0.186 (0.243)	68%	0.272 (0.355)	0.286 (0.373)	0.134 (0.175)	49%	0.083 (0.108)	30%	0.05
Coconutwater	0.044 (0.149)	29%	0.284 (0.371)	71%	0.398 (0.520)	0.410 (0.535)	0.168 (0.219)	42%	0.099 (0.129)	25%	
Rice	0.069 (0.107)	33%	0.112 (0.146)	59%	0.189 (0.247)	0.198 (0.258)	0.100 (0.130)	52%	0.081 (0.105)	43%	
CD (P≥ 0.05)	**0.09**										

Note: Acetone extracted pigment was used.
Values in parenthesis indicate the quantity of pigment

Table 3. Effect of different temperatures on extracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, Coconut water and rice

Medium used for growth of pigment	Heating Temperature							
	Control	63°C/30min		100°C/10 min		121°C/15 min		CD(P≥ 0.05)
	Extra-cellular Pigment (µg/g of dry cell mass)	Extra-cellular Pigment (µg/g of dry cell mass)	Percent retention	Extra-cellular Pigment (µg/g of dry cell mass)	Percent retention	Extra-cellular Pigment (µg/g of dry cell mass)	Percent retention	
MYEB	0.186 (0.243)	0.126 (0.164)	67%	0.082 (0.107)	44%	0.046 (0.060)	25%	0.06
Coconut water	0.201 (0.262)	0.130 (0.169)	65%	0.073 (0.095)	36%	0.040 (0.052)	20%	
Rice	0.160 (0.209)	0.148 (0.193)	92%	0.083 (0.108)	40%	0.051 (0.066)	32%	
CD (P≥0.05)	**0.08**							

Note:

- Acetone extracted pigment was used
- Values in parenthesis indicate the quantity of pigment

Table 4. Effect of different temperatures on intracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, Coconut water and rice

Medium used for growth of pigment	Heating Temperature							
	Control	63°C/30 min		100°C/10 min		121°C/18 min		CD(P≥ 0.05)
	Intracellular Pigment (µg/g of dry cell mass)	Intracellular Pigment (µg/g of dry cell mass)	Percent retention	Intra-cellular Pigment (µg/g of dry cell mass)	Percent retention	Intra-cellular Pigment (µg/g of dry cell mass)	Percent retention	
MYEB	0.233 (0.304)	0.221 (0.288)	95%	0.121 (0.158)	52%	0.074 (0.096)	32%	0.06
Coconut water	0.472 (0.616)	0.310 (0.405)	67%	0.140 (0.183)	30%	0.081 (0.105)	17%	
Rice	0.372 (0.486)	0.178 (0.232)	48%	0.106 (0.138)	28%	0.060 (0.091)	15%	
CD (P≥0.05)	0.08							

Note:

- Acetone extracted pigment was used
- Values in parenthesis indicate the quantity of pigment

solution showed maximum stability at neutral pH than under acidic and alkaline conditions.

Similarly Kaur *et al.,* (2009) studied the effect of pH of the methanolic solution of the extracted intracellular pigment of Rhodotorula rubra mtcc1446 obtained from MYEB: coconut water (50:50) medium by adjusting to 5, 6 and 7 using 0.1N NaOH and HCl. This pigment solution showed its stability till 1h at pH 5, pH 6 and pH 7. At pH 7 it showed maximum stability with no change in absorbance till 48h (0.705 OD/ml to 0.702 OD/ml) as 99% residual colour remained in the solution. Maximum decrease was observed at pH 5 (0.705 OD/ml to 0.439 OD/ml) with 62% residual colour in the solution. They concluded that this pigment solution is having maximum stability at neutral pH than under acidic conditions.

Effect of different temperatures on extra and intracellular pigment of *Rhodotorula minuta* RAI$_3$ from sterile MYEB, coconut water and rice

It was interesting to know that, intensity of pigment decrease was more rapid in intracellular at laboratory pasteurization A_{520} (0.310 to 0.178) followed by boiling A_{520} (0.140 to 0.096) and sterilization temperatures A_{520} (0.071 to 0.064). The decrease in the intensity of extracellular pigment was slightly lesser compared to intracellular pigment at lab pasteurization A_{520} (0.148 to 0.126), boiling A_{520} (0.083 to 0.073) and sterilization temperature A_{520} (0.051 to 0.046). The media had no effect on heat stability of pigment. Heat treatment had negative effect on intensity of intracellular pigment compared to extracellular pigment (Fig.1).

On par with the present study present study, Kaur et al., (2009) subjected alcoholic extracted pigment solution of *Rhodotorula rubra* to heat treatment from 700-1000C for 15 minutes and measured by taking OD at 360 nm. It was shown that colour decrease was more rapid in intracellular (0.705 OD/ml to 0.36 0D/ml) pigment extracted from submerged fermentation than in intracellular pigment (0.41 OD/ml to 0.16 0D/ml) extracted by solid-state fermentation than extracellular pigment extracted in submerged fermentation (0.28OD/ml to 0.090D/ml).

References

Aksu,-Z., Eren,-A-T 2007. Production of carotenoids by the isolated yeast of *Rhodotorula glutinis. Biochemical Engineering Journal*, **35**:107–11.

Costa HL, Martelli da silva IM and Pomeroy D. 2005.Production of β-carotene by a Rhodotorula strain. *Biotechnol Lett*, **9**: 373-375.

Downham, A. And P. Collins, 2000. Colouring our foods in the last and next millennium. *International Journal Food Science and Technology*, **35**: 5-22.

Frengova, G.I., Simova, D., Beshkova, D.M., 2003. Carotenoid production by lactose- negative yeasts co-cultivated with lactic acid bacteria in whey ultrafiltrate.Zeitschrift für Naturforschung. **58c**: 562–567.

Hammond BR, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, Edwards RB, Snodderly DM.2005. Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci;* **38(9):**1795–1801.

Iriani Rm, Adilma Rp, Scamparini, Delia B, Rodriguez A. 2005.Selection and characterization of carotenoid-producing yeasts from Campinas region. *Brazilian Journal Microbiology,* **40**: 2985-2991.

Kaur, B., D. Chakraborty and H. Kaur., 2009.Production and stability analysis of yellowish pink pigments from *Rhodotorula rubra* MTCC 1446. *Internet Journal Microbiology*, **7:**1.

Latha, B.V., K. Jeevaratnam, H.S. Murali and K.S. Manja, 2005. Influence of growth factors carotenoid pigmentation of Rhodotorula glutinis DFR-PDY from natural source. *Indian Journal Biotechnology*, **4**: 353-357.

Martin A, Lu C, and Patel T., 1993. Growth parameters for the yeast *Rhodotorula rubra* grown in peat extracts. *J. Ferm Bioeng.* **76**: 321–325.

Postgate J. 1994."The Outer Reaches of Life", Cambridge University Press, 132-134, Wikipedia free encyclopedia.