

SHELF LIFE STUDIES OF CAROTENOID PIGMENTS PRODUCED FROM *RHODOTORULA MINUTA*

ABSTRACT:

Aim: The present study aimed to study the storage stability of carotenoid pigment (extra cellular and intracellular) extracted from *Rhodotorula minuta* grown in Malt Yeast Extract Broth (MYEB), coconut water and rice was stored for a period of 15 days at ambient temperature (29⁰C) and refrigeration temperature i.e. 4⁰C and change of optical density was measured by taking absorbance at 520 nm for every 5 days.

Methods and Results: Characterized *Rhodotorula minuta* RAI₃ obtained from air of dairy environment was used in the present study for shelf life study of the pigment. The yeast cultures were maintained as slant cultures on Malt Yeast Extract Agar (MYEA) and working cultures in Malt Yeast Extract Broth (MYEB) with incubation at 30⁰C/3-5 days. Colour was stable both at ambient temperature (29⁰C) and refrigeration temperature (4⁰C) up to 15 days of the study with A520 of 0.420, 0.140, 0.10 and 0.090 and 0.412, 0.320, 0.270 and 0.189 extracellular in MYEB, coconut water and rice.

Conclusion: Storage stability for 15 days both at ambient temperature (29⁰C) and refrigeration temperature (4⁰C) was noticed during storage of the extra and intracellular pigment respectively of *Rhodotorula minuta* RAI₃

Significance and Impact of the Study: The present study was taken up to identify the stability of extra and intracellular pigments extracted from

Rhodotorula minuta which was observed both at ambient temperature (29°C) and refrigeration temperature (4°C) up to 15 days of storage.

KEY WORDS: Carotenoids, Stability, Extracellular, Intracellular pigment, optical density.

1. INTRODUCTION

Colours are the vital constituents and probably the first characteristic properties observed by human senses (Pattanaik et al., 1997). Colour of the commercial products play a vital role to attract the consumers and also represent the quality of the products (Shivalkar Yadav and Prabha, 2014). Now-days commercial markets are reigned by synthetic colorants some of which are toxic, carcinogenic causing severe damage even to vital organs (Duran et al., 2002). This has ignited the zeal for development and application of eco-friendly and economical pigments from natural resources. The various sources of natural pigments are ores, microbes, insects, and plants. Microbes have immense potential to produce various pigments like carotenoids, monascins, violacien and flavins (Duffose, 2006). More specifically, bacteria has the potential to produce different pigments in presence of cheap raw material supplemented to the production medium, that can radically shrink the cost of industrial production (Joshi et al., 2003; Venil et al., 2009; Ahmad et al., 2012). Moreover the microbial pigments are stable, soluble than those derived from plant and animals (Jiang et al., 2005; Gunasekaran and Poorniammal, 2008). Deb and Madhugiri, (2012), used apple pomace as cheap carbon source for production of pigment by *Micrococcus flavus*.

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.

As colour is an important attribute to gain consumer acceptance, adding natural colour to dairy products like flavoured milk, ice cream, burfi, has become a common practice in recent years. The huge international market for carotenoids has been met mainly by synthetic carotenoids with similar structures as natural carotenoids. However due to the possible toxicity of synthetic colours natural colouring alternatives have been increasingly sought (Yadav et al., 2014).

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, 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.

Microbial synthesis offers a promising method for production of carotenoids. This explains the increasing interest in production of microbial carotenoids as alternative for synthetic food colourants. Several algae (*Dunaliella*, *Dictyococcus* and *Haematococcus*), bacteria (many species of eubacteria in addition to halobacteria in archaeobacteria), some filamentous fungi (belong to lower fungi and Ascomycetes), yeasts (*Cryptococcus*, *Phaffia*, *Rhodospiridium*, *Rhodotorula*, *Sporidiobolus*, and *Sporobolomyces*) are reported to produce carotenoid. The major carotenoid pigments obtained by biotechnological methods are torularhodin, β -carotene, and torulene produced in various concentrations by *Rhodotorula* yeasts and astaxanthin from *Phaffia rhodozyma* or the green alga *Haematococcus pluvialis* (Ungureanu et al., 2011).

Kaur et al., (2009) studied the heat stability of carotenoids was studied by heating alcoholic extracts to 70 -100⁰C for 15 min and optical density measured at 360nm. It was observed that colour decrease was more rapid in intracellular OD of *R. rubra* 0.705/ml to 0.36 /ml

Latha and Jeevaratnam (2010) found the storage stability of carotenoids of *Rhodotorula glutinis* DFR-PDY obtained from organic solvents was studied by using two solvents such as petroleum ether (non polar) and acetone (polar) at different temperatures i.e. 4⁰C, ambient temperature and 60⁰C and in the presence/absence of light. At 4⁰C up to 90 days of storage in petroleum ether 35% stability was noticed while in case of acetone only 10% stability was noticed.

Overall stability of carotenoids of *Rhodotorula glutinis* DFR-PDY was more at 4°C at ambient temperature and very poor at 60°C.

In the present study the extracted pigment from *Rhodotorula minuta* was subjected to storage stability for a period of 15 days at ambient temperature (29°C) and refrigeration temperature i.e. 4°C

2. MATERIALS AND METHODS:

2.1 Cultures and their maintenance:

Characterized *Rhodotorula minuta* RAI₃ obtained from air of dairy environment was used in the present study for shelf life study of the pigment. The yeast cultures were maintained as slant cultures on Malt Yeast Extract Agar (MYEA) and working cultures in Malt Yeast Extract Broth (MYEB) (Kaur et al., 2009) broth with incubation at 30°C/3-5 days.

2.1.1 Modified Malt Yeast Extract Agar – MYEA (Kaur et al., 2009)

Malt Extract	-	3.0g
Peptone	-	5.0g
Yeast Extract	-	3.0g
D-glucose	-	10.0ml
Agar-agar	-	15.0g
Distilled water	-	1000ml
pH	-	6.0

Dissolved malt extract, peptone, and yeast extract in distilled water by steaming and adjusted the pH to 6.0. Added D-glucose and agar & dissolved by steaming and sterilized by autoclaving at 121 °C for 15mins. At the time of pouring adjusted to pH 3.5 using 1.6ml of 10% filter sterile lactic acid to 100ml of agar.

2.1.2 Modified Malt Yeast Extract Broth (MYEB) (Kaur, 2009)

Malt yeast extract broth was prepared as mentioned in MYEA (3.3.5) except agar and transferred to sterile tubes as required and sterilized at 121⁰C /15min.

2.2 Growth study of isolates in broth and solid substrate

According to the reviewed literature scientists have used several media for pigment production by *Rhodotorula*. Based on the information the present study was carried out using MYEB as synthetic medium, coconut water as natural liquid medium and rice as a solid substrate. Screened *Rhodotorula* isolates for pigment production were grown in sterile MYEB, coconut water, 50% coconut water : 50%MYEB, 50% whey : 50% coconut water, whey, bengal gram dhal (2 part of dhal soaked in 1 part of tap water, soaked for 10 min and sterilized by autoclaving) and rice were inoculated at 1% level for pigment production. The incubation condition provided was 30⁰C/ 9 days. The Direct Microscopic Count was determined once in 3 days up to 9 days. The media that showed the

presence of pigment visually were further used for the production of pigment by selected isolates of *Rhodotorula*.

2.3 Production and extraction of pigment:

R.minuta RA₁₃, 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 30⁰C for 3rd, 6th and 9 days respectively (Kaur et al., 2009).

2.4 Pigment extraction method

Extraction of extracellular and intracellular pigments from *R.minuta* RA₁₃, *R.acheniorum* RC₂, *Rhodotorula* sp RA₂ and *Rhodotorula* sp RY₁ was carried out using the following flow chart (Peterson *et al.*, 1953).

EXTRACELLULAR PIGMENTS FROM RHODOTORULA SPECIES

Inoculated Rhodotorula into 100 ml of culture media MYEB / Coconut water /

Rice



Centrifuged at 6000 rpm for 10 min and filtered



Separated the supernatant for Extracellular Pigment

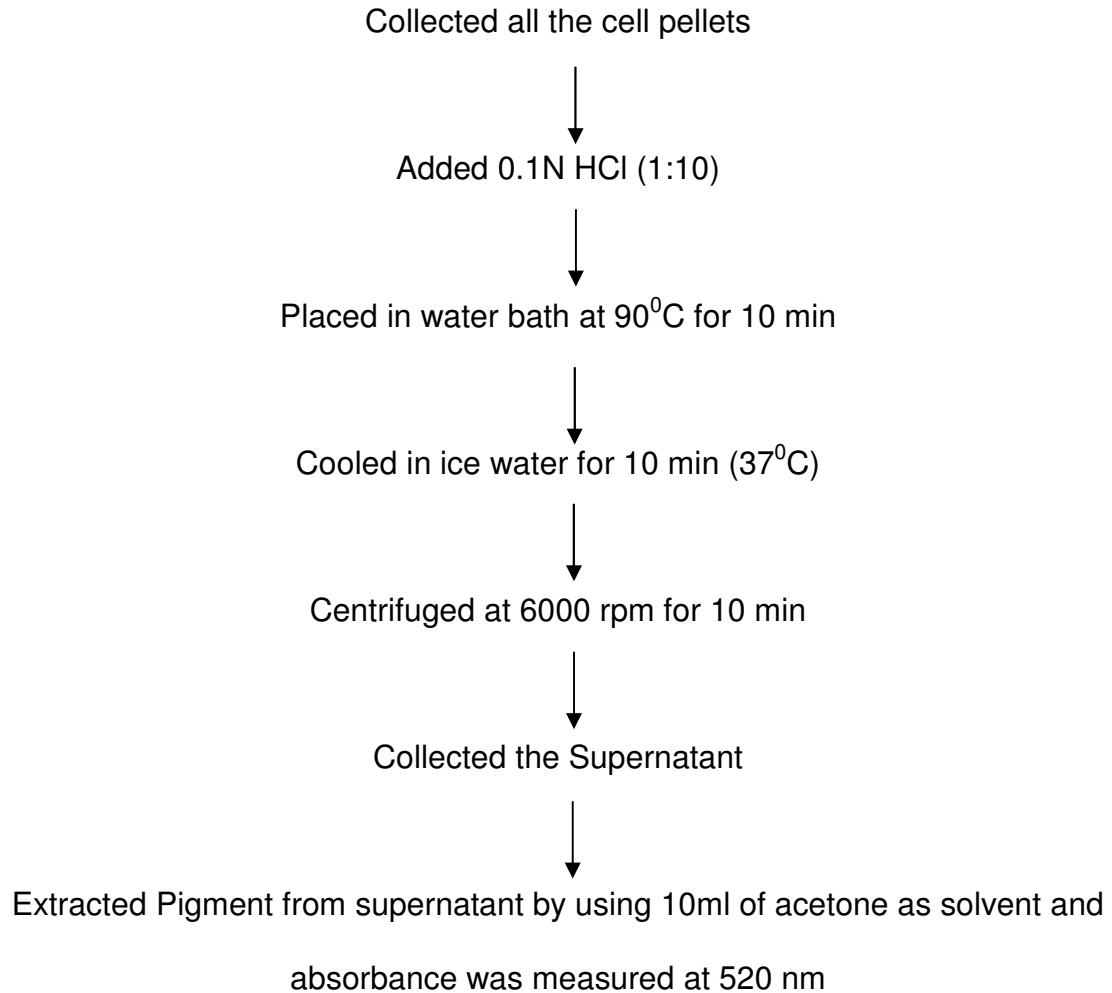


Filter sterilized the supernatant



Extracted Pigment from supernatant by using 10ml of acetone as solvent and
absorbance was measured at 520 nm.

INTRACELLULAR PIGMENTS FROM RHODOTORULA SPECIES



The yield of the pigment was calculate according to the following formula

$$\text{Carotenoid yield (ug/g of dry cell mass)} = \frac{A_{520}(\text{Absorption at 520nm}) \times \text{volume of the acetone}}{\text{Volume of the sample} \times 0.17}$$

2.5 Shelf life study of pigment:

Stability of the acetone extracted pigment solution (extra cellular and intracellular) was stored for a period of 15 days at ambient temperature (29⁰C) and refrigeration temperature i.e. 4⁰C and change of optical density was measured by taking absorbance at 520 nm for every 5 days.

3. RESULTS:

Storage stability of extra and intracellular pigment of *Rhodotorula minuta* RAI₃ obtained from sterile MYEB, coconut water and rice at ambient temperature.

The extracellular pigment extracted from MYEB, coconut water and rice at ambient temperature showed retainment of 69%, 70% and 82% upto 5th day of storage. Later during 10th day, the intensity of pigment decreased nearby 13%, 24% and 13% and on 15th day of storage, intensity of pigment reduced to 9% each in broth and coconut water respectively, while rice pigment showed 11% compared with 10th day of storage. The retainment percent of extracellular pigment was more in rice extracted pigment followed by coconut water and MYEB. While the intracellular pigment stored at ambient temperature showed retainment of 97%, 68% and 48% on 5th day and latter the intensity of pigment decreased nearby 25%, 11% and 11% on 10th day and further storage up to 15 days showed reduction in intensity of pigment by 24%, 17% and 16%. The percent of intracellular pigment retained was more in MYEB extracted pigment followed by coconut water and rice (Tables 1 and 2). Statistically

significant difference was noticed among storage, media and ambient temperature.

Storage stability of extra and intracellular pigment of *Rhodotorula minuta* RAI₃ obtained from sterile MYEB, coconut water and rice in refrigeration temperature

The extracellular pigment extracted from MYEB, coconut water and rice stored at refrigeration temperature for 5 days showed percent retention of 70%, 72% and 85% respectively. Later during 10th day of storage, the intensity of pigment decreased nearby 3%, 6 and 10% compared to storage on 5th day. On 15th day of storage reduction in intensity of pigment was found to be 7, 16 and 13% compared with 10th day. The retainment percent of extra cellular pigment was more in rice extracted pigment followed by coconut water and MYEB (Tables 3 and 4).

While the intracellular pigment extracted from MYEB, coconut water and rice stored at refrigeration temperature showed percent retention in *Rhodotorula minuta* RAI₃ of 90%, 70% and 50% on 5th day and latter the intensity of pigment decreased by 4%, 9% and 17% respectively on 10th day of storage. Further storage up to 15 days showed reduction in intensity of pigment by 10%, 26% and 1%. The percent of intracellular pigment retained was more in MYEB extracted pigment followed by coconut water and rice. Statistically significant difference was noticed among media storage period and temperature.

4. DISCUSSION

Storage stability of extra and intracellular pigments pigment of *Rhodotorula minuta* RAl₃ from sterile MYEB, coconut water and rice.

Acetone extracted pigment solution of *Rhodotorula minuta* was stored at ambient temperature (29⁰C) and refrigeration temperature (4⁰C) for 15 days, and change of optical density was measured by taking absorbance at 520 nm. It was shown that colour was stable both at ambient temperature (29⁰C) and refrigeration temperature (4⁰C) up to 15 days of the study with A520 of 0.420, 0.140, 0.10 and 0.090 and 0.412, 0.320, 0.270 and 0.189 extracellular in MYEB, coconut water and rice.

On par with the above study, Latha and Jeevaratnam (2010) carried out the stability of the pigment of *Rhodotorula glutinis* DFR-PDY in different solvents at OD of 520nm for 3 months. Stability was studied in acetone and in petroleum ether (4⁰C, 25⁰C Room Temperature, 60⁰C) in three different temperatures, 4⁰C, 25⁰C and 40⁰C and in presence of light and in dark.

The pigment showed 10 % stability and 35 % stability in acetone and petroleum ether at 4⁰C respectively compared to ambient temperature and 60⁰C.

5. CONCLUSION:

The colour of extra and intracellular pigment of *Rhodotorula minuta* RAl₃ was stable both at ambient temperature (29⁰C) and refrigeration temperature (4⁰C) up to 15 days of the study extracted from MYEB, coconut water and rice.

CONFLICT OF INTEREST:

No Conflict of Interest declared

Table 1: Storage stability of extracellular pigment of *Rhodotorula minuta* RAl₃ obtained from sterile MYEB, coconut water and rice at ambient temperature (29°C)

Medium used for growth of pigment	Storage stability at ambient temperature 29 ^o C							CD(P≥ 0.05)
	Control	5 th		10 th		15 th		
	Extracellular Pigment	Extracellular Pigment	Percent retention	Extracellular Pigment	Percent retention	Extracellular Pigment	Percent retention	
MYEB	0.186 (0.243)	0.128 (0.167)	69%	0.104 (0.135)	56%	0.088 (0.115)	47%	0.06
Coconut water	0.201 (0.262)	0.140 (0.183)	70%	0.108 (0.141)	54%	0.090 (0.117)	45%	
Rice	0.160 (0.209)	0.132 (0.172)	82%	0.111 (0.145)	69%	0.094 (0.122)	58%	
CD (P≥0.05)	0.08							

Note:

Acetone extracted pigment was used

Values in parenthesis indicate the quantity of pigment

Table 2: Storage stability of intracellular pigment of *Rhodotorula minuta* RAl₃ obtained from sterile MYEB, coconut water and rice at ambient temperature (29°C)

Medium used for growth of pigment	Storage stability at ambient temperature 29 ⁰ C							CD(P≥ 0.05)
	Control	5 th		10 th		15 th		
	Intracellular Pigment	Intracellular Pigment	Percent retention	Intracellular Pigment	Percent retention	Intracellular Pigment	Percent retention	
MYEB	0.233 (0.304)	0.226 (0.295)	97%	0.169 (0.220)	72%	0.113 (0.147)	48%	0.06
Coconut water	0.472 (0.616)	0.320 (0.418)	68%	0.270 (0.352)	57%	0.189 (0.247)	40%	
Rice	0.372 (0.486)	0.178 (0.232)	48%	0.138 (0.180)	37%	0.101 (0.132)	21%	
CD (P≥0.05)	0.08							

Note:

Acetone extracted pigment was used

Values in parenthesis indicate the quantity of pigment

Table 3: Storage stability of extracellular pigment of *Rhodotorula minuta* RAI₃ obtained from sterile MYEB, coconut water and rice at refrigeration temperature (4°C)

Medium used for growth of pigment	Storage stability at refrigeration temperature 4 ^o C							
	Control	5 th		10 th		15 th		CD(P≥ 0.05)
	Extracellular Pigment	Extracellular Pigment	Percent retention	Extracellular Pigment	Percent retention	Extracellular Pigment	Percent retention	
MYEB	0.186 (0.243)	0.131 (0.179)	70%	0.126 (0.164)	67%	0.111 (0.145)	60%	0.07
Coconut water	0.201 (0.262)	0.144 (0.188)	72%	0.132 (0.172)	66%	0.101 (0.132)	50%	
Rice	0.160 (0.209)	0.136 (0.177)	85%	0.120 (0.156)	75%	0.099 (0.129)	62%	
CD (P≥0.05)	0.09							

Note:

- Values in parenthesis indicate the quantity of pigment

Table 4: Storage stability of intracellular pigment of *Rhodotorula minuta* RAl₃ obtained from sterile MYEB, coconut water and rice at refrigeration temperature (4°C)

Medium used for growth of pigment	Storage stability at refrigeration temperature 4 ⁰ C							
	Control	5 th		10 th		15 th		CD(P≥ 0.05)
	Intracellular Pigment	Intracellular Pigment	Percent retention	Intracellular Pigment	Percent retention	Intracellular Pigment	Percent retention	
MYEB	0.233 (0.304)	0.211 (0.275)	90%	0.201 (0.262)	86%	0.178 (0.232)	76%	0.07
Coconut water	0.472 (0.616)	0.331 (0.432)	70%	0.289 (0.377)	61%	0.165 (0.215)	35%	
Rice	0.372 (0.486)	0.188 (0.245)	50%	0.159 (0.207)	33%	0.120 (0.156)	32%	
CD (P≥0.05)	0.09							

Note: Acetone extracted pigment was used

➤ Values in parenthesis indicate the quantity of pigment

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