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

ABSTRACT:

Aims: The present study aimed to study the storage stability of carotenoid pigment (extra cellular and intracellular) extracted from *Rhodotorula minuta* grown in MYEB, coconut water and rice was stored for a period of 15 days at room temperature (29^0C) and refrigeration temperature i.e. 4^0C and change of optical density was measured by taking absorbance at 520 nm for every 5 days.

Methods and Results: *Rhodotorula minuta* RAI_3 which was characterized and maintained in Department of Dairy Microbiology, KVAFSU 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 broth with incubation at 30^0C/3-5 days. Colour was stable both at room temperature (29^0C) and refrigeration temperature (4^0C) upto 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 room temperature (29^0C) and refrigeration temperature (4^0C) was noticed during storage of the extra and intracellular pigment respectively of *Rhodotorula minuta* RAI_3

Significance and Impact of the Study: This study provides insights stability of extra and intracellular pigments was observed both at room temperature (290C) and refrigeration temperature (40C) upto 15 days of storage

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

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1. INTRODUCTION

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 & cataract and enhancement of immune response. (Costa et al*.,* 2005; Iriani et al., 2005).

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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.

Kaur (2009) studied the heat stability of carotenoids was studied by heating alcoholic extracts to 70 -100^{0}C 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 (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 40^{0}C, room temperature and 60^{0}C and in the presence/absence of light. At 4^{0}C upto 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^{0}C & room temperature and very poor at 60^{0}C.

In the present study the extracted pigment from *Rhodotorula minuta* was subjected to storage stability for a period of 15 days at room temperature (29^{0}C) and refrigeration temperature i.e. 4 ^{0}C

2. MATERIALS AND METHODS:

2.1 Cultures and their maintenance:

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Characterized *Rhodotorula minuta* RAI_3 maintained in Department of Dairy Microbiology 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 broth with incubation at 30^0C/3-5 days.

2.2 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 30^0C for 3rd, 6th and 9 days respectively **(**Kaur et al.**,** 2009**)**.

2.3Shelf life study of pigment:

Stability of the acetone extracted pigment solution (extra cellular and intracellular) was stored for a period of 15 days at room temperature (29^0C) and refrigeration temperature i.e. 4^0C 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* RAI_3 obtained from sterile MYEB, coconut water and rice at room temperature.

The extracellular pigment extracted from MYEB, coconut water and rice at room temperature showed retainment of 69%, 70% and 82.5%. Later during 10th day of storage, the intensity of pigment decreased nearby 13%, 24% and 13.5% compared to storage on 5th day and on 15th day of storage reduction in intensity of pigment was found to be 9%, 12% and to 11% compared with 10th day The retainment percent of extracellular pigment was more in rice extracted pigment followed by coconut water and

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MYEB. While the intracellular pigment stored at room temperature showed retainment of 97%, 67% and 48% on 5th day and latter the intensity of pigment decreased nearby 24%, 11% and 11% on 10thday and further storage upto 15 days showed reduction in intensity of pigment by 24%, 17% and 10%.The percent of intracellular pigment retained was more in MYEB extracted pigment followed by coconut water and rice (Table 1 and 2 and Fig 1). Statistically significant difference was noticed among storage, media and room temperature.

Storage stability of extra and intracellular pigment of *Rhodotorula minuta* RAI_3 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 2%, 6 and 10% compared to storage on 5th day. On 15th day of storage reduction in intensity of pigment was found to be 10, 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 (Table 3 and 4and Fig 2).

While the intracellular pigment extracted from MYEB, coconut water and rice stored at refrigeration temperature showed reduction in *Rhodotorula minuta* RAI_3 of 10%, 30% and 49% on 5th day and latter the intensity of pigment decreased by 4%, 9% and 8% respectively on 10th day of storage. Further storage upto 15 days showed reduction in intensity of pigment by 10%, 26% and 11%.The percent of intracellular

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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* RAI3 from sterile MYEB, coconut water and rice.

Acetone extracted pigment solution of *Rhodotorula minuta* was stored at room temperature (29^0C) and refrigeration temperature (4^0C) 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 room temperature (29^0C) and refrigeration temperature (4^0C) upto 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 (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 (4^0C, 25^0C- RT, 60℃) in three different temperatures, 4℃, 25℃ and 40℃ and in presence of light and in dark.

The pigment showed 10 % stability and 35 % stability in acetone and petroleum ether at 4^0C respectively compared to room temperature and 60^0C.

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Table 1: Storage stability of extracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, coconut water and rice at room temperature (29^0C)

Medium used for growth of pigment	Storage stability at room temperature 29^0C							
	Control	5th		10th		15th		CD(P≥ 0.05)
	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

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Table 2: Storage stability of intracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, coconut water and rice at room temperature (29^0C)

Medium used for growth of pigment	Storage stability at room temperature 29^0C							
	Control	5th		10th		15th		CD(P≥ 0.05)
	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%	
Coconut water	0.472 (0.616)	0.320 (0.418)	68%	0.270 (0.352)	57%	0.189 (0.247)	40%	**0.06**
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

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Table 3: Storage stability of extracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, coconut water and rice at refrigeration temperature (4^0C)

Medium used for growth of pigment	Storage stability at refrigeration temperature 4^0C							
	Control	5th		10th		15th		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%	
Coconut water	0.201 (0.262)	0.144 (0.188)	72%	0.132 (0.172)	66%	0.101 (0.132)	50%	**0.07**
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* RAI_3 obtained from sterile MYEB, coconut water and rice at refrigeration temperature (4^0C)

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Medium used for growth of pigment	Storage stability at refrigeration temperature 4^0C							
	Control	**5th**		**10th**		**15th**		**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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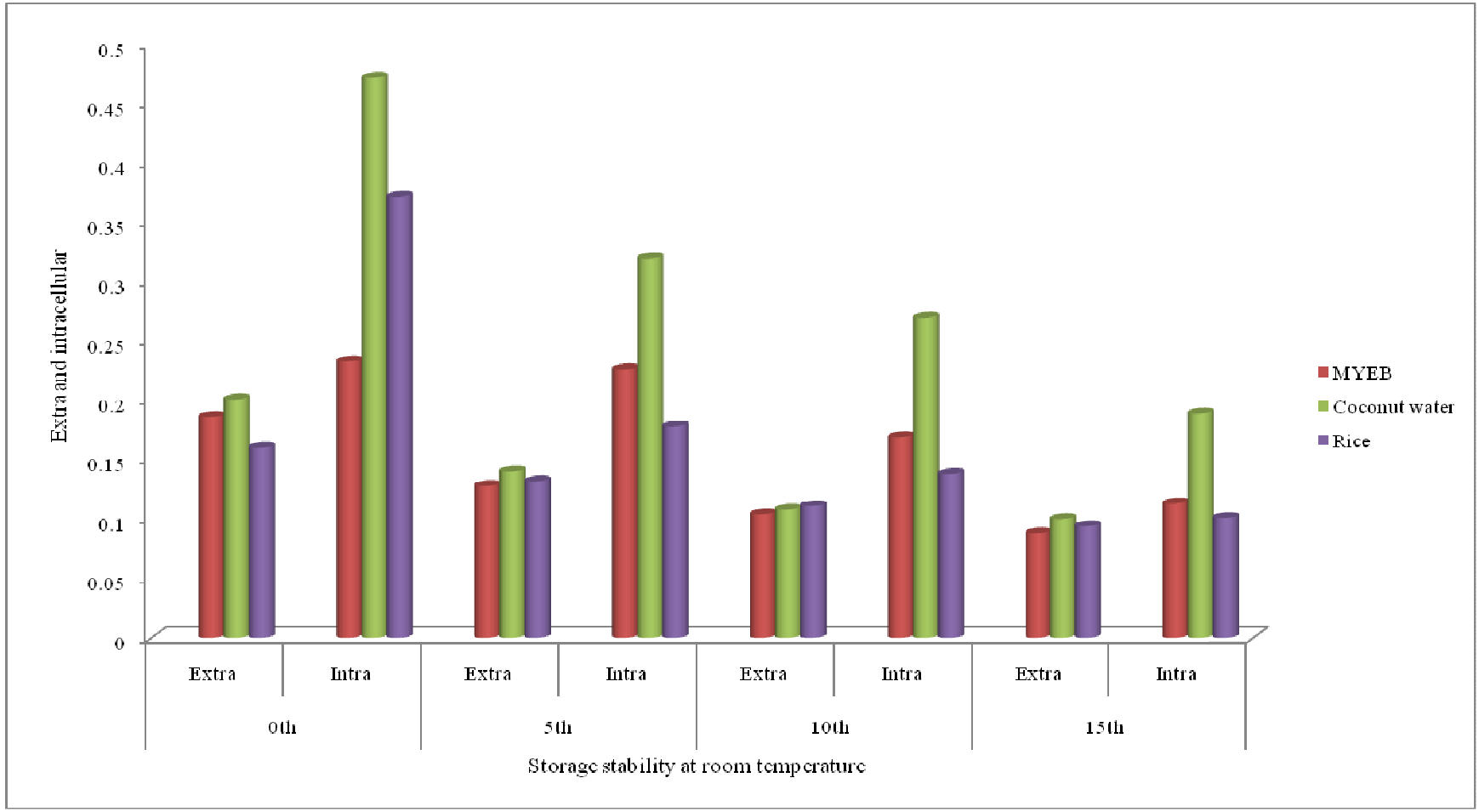

Fig. 1 : Shelf life studies of extra and intracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, coconut water and rice at room temperature

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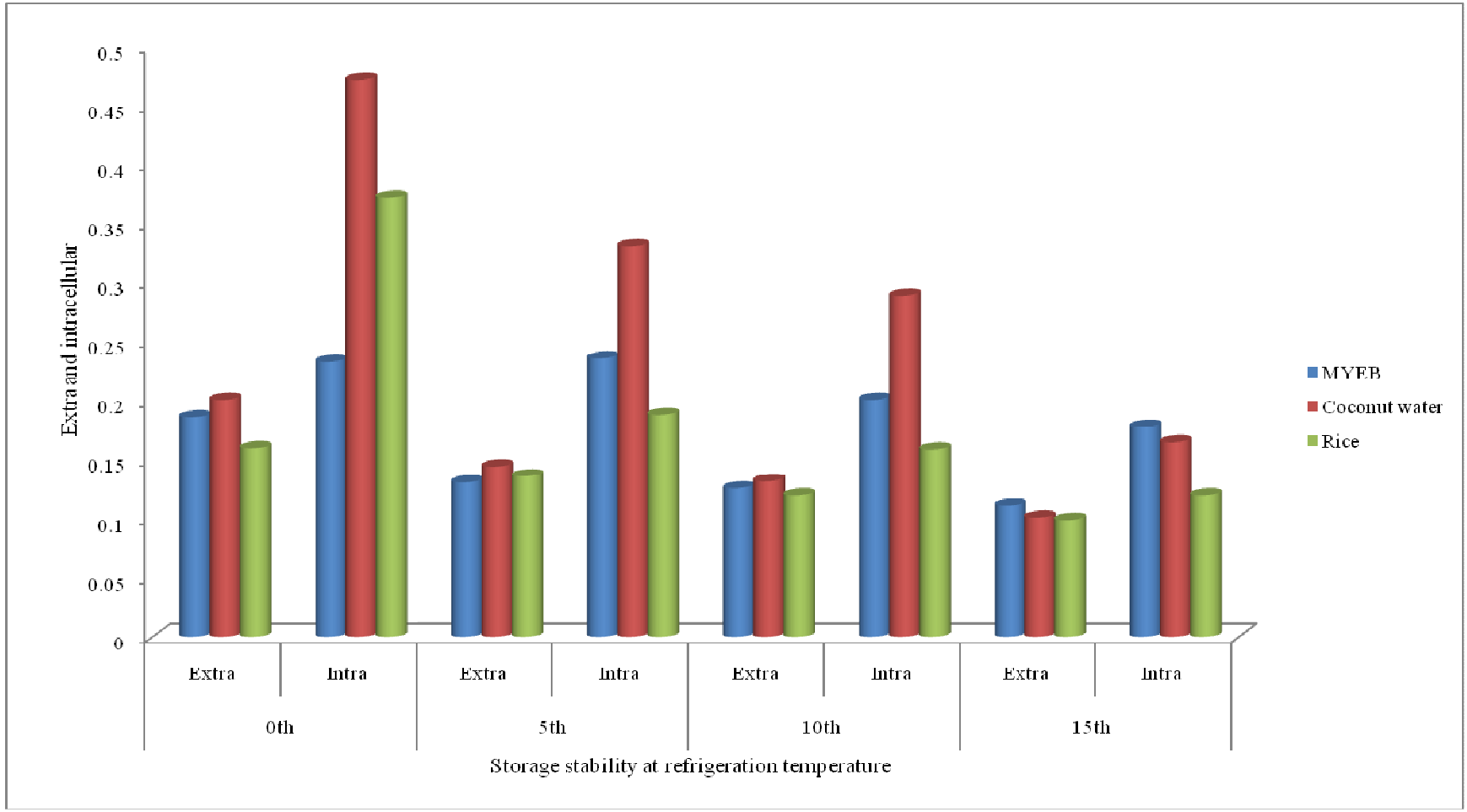

Fig. 2 : Storage stability of extra and intracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, Coconut water and rice at refrigeration temperature

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