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Enhancing carotenoid production of marine *Rhodotorula mucilaginosa* YM with fruit peel extract recycling market waste using central composite design

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Abstract
Carotenoid produced by microorganism utilizing low-cost substrates offers a promising eco-friendly and cost-effective approach used alternative for synthetic colourants. Vegetable and fruit processing wastes are the rich sources of carbohydrates, starch, cellulose, soluble sugars, minerals and organic acids. In the current study with fruit waste extract medium, carotenoid production was optimized using software tools, Central Composite design. The effect of pH, inoculum volume (% v v^{-1}) and incubation time (h) on carotenoid production by marine isolate *Rhodotorula mucilaginosa* YM utilizing fruit peel waste extract as sole carbon source containing peels of oranges (11% w v^{-1}), core of pineapples (24% w v^{-1}), peels papaya (29% w v^{-1}) and flesh of watermelons (36% w v^{-1}) was analysed and optimized using Central Composite Design (CCD). The marine isolate *R. mucilaginosa* YM showed enhanced carotenoid production (51.32±0.6 mg L^{-1}) under optimum condition of pH 5.5 with 2% (v v^{-1}) inoculum after 48 h incubation time. HPLC and FT-IR spectrum confirmed that the carotenoid has an astaxanthin compounds and had sun protection properties. Thus, fruit waste medium with optimized process parameters reduces production time, recycles and reutilizes waste for carotenoid production that can be further scaled up.

Keywords: *Rhodotorula mucilaginosa*, carotenoid, central composite design, fruit waste, astaxanthin

Introduction
Carotenoids are natural fat-soluble tetraterpene pigments, which show yellow, orange, red and purple colors. Carotenoids are widely distributed in photosynthetic organisms, few species of archaea and fungi, algae, plants and in many fruits and vegetables [1]. Compared with carotenoid from plant source production of carotenoids from microorganism are more effective due to low cost unaffected by seasonal changes. However, the carotenoids market is still mainly occupied by chemically synthetic products (80–90%), with a much lower portion of natural sources (10-20%). Thus, selecting eco-friendly, economic and robust microorganisms for carotenoid production is focused for extensive research [2].
The use of carotenoids as dietary supplements or active compounds in pharmaceuticals has been shown to delay the onset and significantly reduce the risk of diseases such as atherosclerosis, cataracts, macular degeneration, multiple sclerosis and various types of cancer [3]. Microbial carotenoids also possess antibacterial, anti-ultraviolet (UV) radiation and antifouling mechanisms, and act as an organism defense against predators [4, 5]. These naturally occurring pigments are reflection of the secondary metabolites of microorganisms with great commercial value in food and dairy, cosmetics, pharmaceutical, textile, and dyeing industry [6].
In bacteria, *Flavobacterium* sp, *Erythobacter* sp, *Deinococcus radiodurans*, *Paracoccus zeaanthinifaciens*, *Bradyrhizobium* sp, *Agrobacterium aurantiacum*, *Paracoccus carotinifaciens*, *Halobacterium salinarium*, *Halobacterium sarcina*, *Lactobacillus plantarum* strain CECT7531, *Staphylococcus aureus*, *Thermus thermophiles*, *Erythobacter* sp. and *Micrococcus luteus* are identified to produce various carotenoids [7]. Microalgae species belonging to genus *Chlorella*, *Arthrospira* (*Spirulina*), *Scenedesmus*, *Dunaliella*, *Arthrospira* (*Spirulina*), and *Haematococcus* are considered as the key producers of commercially vital carotenoids [8]. Fungi and yeasts like *Blakeslea trispora*, *Rhodosporidium*, *Sporobolomyces*, *Monascus* sp., *Ashbya gossypi*, *Phycomyces blakesleeanus*, *Candida* sp., *Xanthophyllomyces*

dendrorhous, *Phaffia rhodozyma*, *Rhodotorula* sp. are discovered to have the ability to synthesize carotenoids [9].

Carotenoid biosynthesis from coloured yeasts, e.g. *Rhodotorula* or *Phaffia*, has attracted commercial interest because yeasts are more convenient than algae or fungi for large- scale fermentation, due to their high growth rate and unicellular nature. Also, carotenoids production using yeast can be highlighted by using simple nitrogen and carbon sources for its growth, being easy to adapt on inexpensive substrates. In addition, many of the potential producers are considered GRAS (Generally recognized as safe). For the growth and production of yeast and carotenoid respectively, it is necessary to determine the best culture medium and environmental conditions in order to exploit the potential of the selected strain [10]. The production of carotenoids by fermentation is limited by the processing costs, but the use of agro-industrial by-products as a source of nutrients, can reduce costs and to make possible the industrial production of these compounds [11].

India is positioned as second largest producer of fruits and vegetables in the world. But the wastage quantity of total production is very high, due to the availability of less processing sectors. Vegetable and fruit processing wastes are the rich sources of carbohydrates, starch, cellulose, soluble sugars, minerals and organic acids. Thus, excess production including fruits and vegetable waste after processing can be considered as best suited substrates for solid-state fermentation to produce microbial pigments [12]. Carotenoid biosynthesis depends on the environment in which the producing organism grows. Yield of carotenoids is directly related to the total biomass yield, to keep both high growth rates and high flow carbon efficiency to carotenoids by optimal cultivation conditions is required parameter for attaining the increased productivity. During growth, different types of environmental and physiological stress conditions constantly challenge all organisms [13]. In additional to media components optimization of fermentative conditions is essential to achieve maximizing yield of the desire product. Optimization of operating conditions includes factors such temperature, pH, aeration, agitation, inoculum density and production time. Statistical methodologies such as factorial designs and response surface methodology (RSM) may provide a comprehension of the factors involved in bio production based on experimental trials [14, 15]. One of the most effective methods of RSM of studying the impact of different independent variables and their interactions within the system or method is central composite design (CCD) to fit a model [16]. Several studies have been carried out on extraction of carotenoid from fruits, fruit juice and on optimization of media composition using statistical tools [17-20]. However, enhancing carotenoid production using yeast strain with fruit waste substrate were not evaluated by statistical approach.

In the consideration of the presented facts of carotenoid and its growing commercial value, this study was focused to find carotenoid-pigment producing strains from ecological niches. Further aimed to step-up the carotenoid production using agro waste as low-cost substrate collect from fruit market, optimizing the fermentation conditions utilizing software tools and analyze the extract carotenoids using HPLC and FT-IR. Additionally this work explores the ability of carotenoids in photo-protection.

Materials and Methods

Isolation of Pigment producing microorganisms

The marine water samples were collected from Thiruchendur (8°29'37.7"N 78°07'39.5"E) in Thoothukudi district, Tamil Nadu. The samples were collected in sterile containers serially diluted and spread plated on Yeast Malt Agar plate containing chloramphenicol 100 mg L^{-1} for isolation of yeast. The plates were incubated at 25 °C for 48 h and constantly observed for 120 h [21]. The colonies showing discrete non-diffusible pigmented morphology were selected purified, transferred to agar slants and stored at 4 °C until further analysis.

Screening was performed based on estimation of total carotenoid by Asker *et al.* using direct colonies method [22]. The colonies (approximately 50 mg) were suspended in 200 µL DMSO and kept at 50 °C in the dark (5–10 min) with consistent agitation to initiate lysis of the cells. The lysed cells were added with an equal volume of methanol, mixed and centrifuged to obtain the carotenoid extract. The total carotenoid was measured spectrophotometrically and expressed as μLg^{-1} of culture [23]. The selected yeast isolate was identified using 18s rRNA sequencing analysis (Exonn Biosciences, Vandalur, Chennai, Tamil Nadu).

Estimation of Total Carotenoid in liquid medium

The liquid medium containing catenoid producing yeast strain were centrifuged at 4000 rpm for 10 min. The cell pellet was washed three times with distilled water, suspended in 200 µL dimethyl sulfoxide (DMSO) and heated at 50 °C for 5-10 minutes. Then equal amount of methanol was added to them and mixed thoroughly. After centrifugation, the solvent was pipetted off as carotenoid extract and extraction was proceeded repeatedly until the cells become grey. The total carotenoid content of the cells was calculated and expressed as volumetric carotenoids (mg L^{-1}) of culture [24].

Production of Carotenoids using low cost Agro waste

The selected strain was analyzed for production of carotenoid in different medium using low cost agro waste as substrate. Molasses based medium was prepared using the composition Molasses (1 g L^{-1}), Yeast extract (6.4 g L^{-1}), Potassium hydrogen orthophosphate (0.5 g L^{-1}), Potassium dihydrogen orthophosphate (0.5 g L^{-1}), Magnesium sulphate (0.2 g L^{-1}) and Calcium chloride (0.05 g L^{-1}) [25]. Medium prepared with vegetable waste includes outer cabbage leaves (15% w v^{-1}), tomato peels (25% w v^{-1}), bean pods (32% w v^{-1}), carrot peels (13% w v^{-1}) and onion peels (15% w v^{-1}). The extract was prepared by adding 2 litres of water to 1 kg of vegetable waste. Then they are heated at 100 °C for 30 minutes filtered and used for experiment. Fruit waste typically containing peels of oranges (11% w v^{-1}), core of pineapples (24% w v^{-1}), peels papaya (29% w v^{-1}) and flesh of watermelons (36% w v^{-1}) were obtained from a fruit juice shop of a local market. The extract was prepared by adding 2 litres of water to 1 kg of fruit waste. Then it was heated to 100 °C for 30 minutes, filtered and used as fermentation medium. The agro wastes based media (50 mL) were taken in separate flask 1% (v v^{-1}) of inoculum was added and incubated at 25 °C on rotatory shaker at 100 rpm [26]. The cells were harvested and the total carotenoids were estimated after 72 h of incubation.

Response surface methodology

The effect of pH, inoculum density (v v^{-1}%) and incubation time (h) on carotenoid production using fruit waste extract was analysed and optimized by Central Composite methodology using the 'Design Expert' software (Version 13.0, Stat-Ease Inc., Minneapolis, USA). In this study, the experimental plan consisted of 20 runs and the independent variables were studied at low (-1) and high (+) values. All the experiments were performed in replicate, and the average of total carotenoid estimated was takes as response R1(Y). The second order polynomial coefficients were calculated and analyzed as follows:

$Y= \beta0 + \beta1\ A + \beta2\ B + \beta3\ C + \beta1\ \beta1\ A^2 + \beta2\ \beta2\ B^2 + \beta3\ \beta3\ C^2 + \beta1\ \beta2\ AB + \beta1\ \beta3\ AC + \beta2\ \beta3\ BC$

Where Y is response variable, β0 is intercept, β1, β2 and β3 are linear coefficients, β1,1, β2,2, and β3,3 is squared coefficient and A, B, C, A^2, B^2, C^2, AB, AC and BC are level of independent variables. Analysis of Variance (ANOVA) was determined by statistical evaluation of the model. This analysis included the Fisher's F-Test (overall model significance), its associated probability (F), correlation coefficient (R), determination coefficient (R^2) which measures the goodness of fit of regression model. For each variable, the quadratic models were represented as counter plots (3D) and response curves were generated using STATISTICA software (Stat Soft Inc., USA).

HPLC and FTIR analysis

The extraction and determination of carotenoid was performed as previously described by Kanzy *et al.* [24]. In HPLC (Flexar FX UV Det-2.1, USA) analysis, 10 µL of carotenoid extract was eluted with methanol: water (9:1) solvent system with silica column for run time of 30 minutes. The structure of three fractions were determined using FTIR (Shimadzu FTIR 84000 S, Japan) absorption spectra using KBr [27].

Determination of Sun protection factor

The effectiveness of protection against UV light of carotenoid extract was determined *in vitro* with a UV-visible spectrophotometer (Shimadzu UV-2400 PC series, Japan). The sun protection factor (SPFs) of crude pigment extract was determined at a concentration of 1000 ppm. At the intervals of 5 nm from 290-320 nm the average absorbance was measured and the constant normalized product function was referred by Sayre *et al.* [28]. The SPF value was calculated using the Mansur equation [29].

Results and Discussion

Isolation and Identification of Carotenoid producing yeast strain

In this study production of carotenoid from marine *R. mucilaginosa* YM was investigated. Among the 570 yeast strains screened isolate strain YM was selected for further studies. For identification of strain YM, 18s rRNA sequencing analysis was performed. The Blast result of partial sequence of 18S rRNA gene showed that the isolate exhibit 100% similarity to *Rhodotorula mucilaginosa.* The 18s rRNA partial sequence of *Rhodotorula mucilaginosa* YM was deposited in GenBank under the accession number MZ008005. The genus *Rhodotorula* having energy production by oxidative pathway can grow in medium even without vitamins includes twenty-two species generally. They are widely explored due to pigment production of yellow, orange or reddish colour that has immense application in pharmaceuticals, cosmetics and food industries. Similarily, the *Rhodotorula* sp. is heterogenous yeast isolated from soil, food and marine source [30-31].

Carotenoid production using different Agro-waste

The ability of *R. mucilaginosa* YM to grow and produce carotenoids on various agro waste substrates were analyzed using molasses based medium, vegetable waste extract and fruit waste extract. The total carotenoid production in agro waste substrate shows enhanced production than the basic nutrient medium. After 72 h of incubation, medium with fruit waste extract (FWE) shows higher yield of 36.18± 0.2 mg L^{-1} of total carotenoids followed by vegetable waste extract (VWE) and molasses based medium (Fig. 1). The results emphasis that fruit waste enhanced carotenoid production compared with other agro waste. This result coincides with the finding of Tarangini and Mishra [32] have also showed efficient carotenoid production of *R. rubra* with fruit waste extract as sole source of substrate has yield 6.96 mg mL^{-1}. Similarly, the carotenoid production by *Blakeslea trispora* in media containing both fruit and vegetable substrate gave good yield of β-carotene (0.127 mg mL^{-1}), in respect to synthetic media containing nutrient sources [12].

Optimization of Carotenoid production through Central Composite Response Surface design

Optimization studies performed with response surface methodology (RSM) facilitates to evaluate the effect of many factors and investigate its interactions [33]. Central Composite Design was used to optimize the fermentation conditions of total carotenoid production of *R. mucilaginosa* YM using Fruit waste extract. Central Composite Design model was designed with three independent variables. The actual experiment was carried out according to the design in 20 runs for up to 88 h. The response of the experimental runs was measured with total carotenoid production (Table 1). The second degree (quadratic poly-nominal regression) model for the response variables were used to study the optimum level of factors and effect of their interactions (Table 2). The fitted equation for the carotenoid production by *R. mucilaginosa* YM by applying is as follows:

$R1 = -117.916 + 33.323\ A + 34.241\ B + 1.361\ C + 0.034\ AB + 0.010\ AC - 0.069\ BC - 3.117\ A^2 - 6.750\ B^2 - 0.012\ C^2$

Where R1 is the Response of Total carotenoids (mg L^{-1}), A is the pH, B is the inoculum density (% v v^{-1}), and C is the incubation time (h). The analysis of variance (ANOVA) was performed through joint test of three independent variables to test the significance of the fit of the equation. The Model F-value of *R. mucilaginosa* YM is 42.75 implying that the model is significant and the chance of F-value this large could occur due to noise is 0.01%. The model p-value is < 0.0001 which indicates that the model was significant. In this study, A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant model terms. The high determination coefficient (R^2) of this model was 0.9747. The Predicted R^2 and Adjusted R^2 of this study is 0.8627 and 0.9519 respectively with the difference less than 0.2. This implies that there is reasonable agreement between Predicted R^2 and Adjusted R^2 (Table 3). For all the independent variables A, B, C the linear, quadratic and cross interactions were checked for significance levels against p

value. The p-value suggests the significance of each coefficient and effect of mutual interaction between the coefficients. The p-value is indirectly proportional to corresponding coefficient. Therefore, smaller the p-value, greater the significance of corresponding coefficient. From the ANOVA it can be observed that all linear, quadratic and cross interaction are highly significant (Table 4). The relation between the response and independent variables can be identified using Response surface. The contour plot shows that effect of variables pH, inoculum density and incubation period have significant effect on production of carotenoid and it is positively interacted with one another. The variation effect of the independent variables on the carotenoid production shown in the perturbation graph concluded that all the variables pH, inoculum volume and incubation time affect the carotenoid production. The normal plot of residual graph shows that data were close to straight line and situated at both sides indicating the model is fairly good (Fig. 2). From the Response surface studies, it can be interpreting that the maximum yield of carotenoid production (51.32±0.6 mg L^{-1}) was produced in medium having pH 5.5 with 2% (v v^{-1}) inoculum volume with 48 h incubation time, also suggesting that the predicted values are good in agreement.

To validate the predicted results, the experimental tests were carried out in replicates. The experiment performed shows that pH 5.5 is optimum for *R. mucilaginosa* YM and inoculum density of 2% (v v^{-1}) reduces the incubation period to 48 h and achieved increased production compared with unoptimized condition. Similarily, the optimum conditions for carotenoid production by *Rhodotorula* sp. RY1801 was found at temperature, 28 °C; pH 5.0; carbon source (10 g L^{-1} glucose) nitrogen source (10 g L^{-1} yeast extract) maximum concentration of 987µg L^{-1} of total carotenoids was obtained [34]. The carotenoids production by yeast strain *R. mucilaginosa* using onion peels and mung bean husk were obtained as pH 6.1 at temperature of 25.8°C with agitation of 119.6 rpm for 84 h had produced 717.35 µg g^{-1} of β-carotene [35]. Whereas, Garuba *et al.* investigated that the pH of 6.5 yield pigment 208.21±2.01µg^{-1} by *Rhodotorula* sp. A21 [19].

Characterization of Carotenoid

HPLC analysis for carotenoid extraction was done using silica column and 90% methanol as mobile phase with run time of 30 minutes (Fig. 3) The peak at retention time (Rt) 1.801 minutes shows high similarity with Hydroxy-astaxanthin (Rt = 1.79 minutes) described by Asker *et al.* under HPLC analytic condition with retention time of Dihydroxy-astaxanthin as 1.34 minutes which have very slight similarity with our peak obtained at 1.269 minutes [22]. Thus, it was concluded that carotenoid extract from *R. mucilaginosa* YM may have Hydroxy-astaxanthin and Dihydroxy-astaxanthin. The FT-IR spectrum of the carotenoid extract was recorded in the range of 300-4500 cm^{-1}. Using FT-IR spectrum, the functional groups can be resolved. The bond and functional group for the peak value was identified using standard IR spectrum (Fig. 4). Characterization experiment carried out with carotenoid of *R. mucilaginosa* YM strain identifies the presence of astaxanthin compound. This result was supported by earlier works where the FTIR spectrum of pure astaxanthin, shows in the region of 1700 –750 cm^{-1}, additionally characteristic peaks of C=O stretching at 1680 cm^{-1}, O–H bending at 3300 cm^{-1}, C=C stretching (aromatic structure) at 1600 $cm^{-1}$and -CH3 stretching at 2847 cm^{-1} [27]. The FTIR spectra showing peak at 3362 cm^{-1} (O-H hydrogen bond), 2970 cm^{-1} (methine C-H stretch) 1736 cm^{-1} (C=O bond), 1380 cm^{-1} (methyl C-H asymmetric band), 1163 cm^{-1} (C-O stretch) and the peak at 950 cm^{-1} (skeletal vibrations) was identified as astaxanthin [26]. It can observe that carotenoid extract has fine C=O stretching vibration at 1662.97 cm^{-1} and two OH bond was found at 3032.60 cm^{-1} and 3061.08 cm^{-1}. Also, aromatic C = C stretching with fine peak was observed at 1495.6535 cm^{-1}. The peak at 2970.82 cm^{-1} and 2924.04 cm^{-1} belongs to C-H stretching. The peak values and functional groups are in accordance with the reference above. Also, the spectrum image shows strong resemblance with FT-IR spectrum of astaxanthin described by Chen *et al.* [36]. This confirms that carotenoid has astaxanthin compounds. The presence of C-S stretching and SO3 symmetric stretching was may be due to use of Dimethyl sulfoxide (DMSO) as solvent for extraction.

Evaluation of Sun Protection Factor

The absorbance value of carotenoid was measured from 290-320 nm at 5 nm interval using UV-Visible spectrophotometer and Sun Protection Factor (SPF) value of *R. mucilaginosa* YM carotenoid was found to be 3.138 (Table 5). Astaxanthin has powerful antioxidant activity and its unique molecular and biochemical messenger properties has implications in treating and preventing skin disease as they have inhibition of collagenases, Matrix metalloproteinase activity, inflammatory mediators, and Reactive Oxygen Species induction, resulting in potent antiwrinkle and antioxidant effects and may prevent UV-induced immunosuppression [37]. Sun screening property was analysed with SPF of 3.138 for carotenoid of R. *mucilaginosa* YM. In similar way pigment of *Virgibacillus salaries* from Panjang Island show SPF value 6, many other bacterial and fungal isolates from Panjang and Karimunjawa exhibit SPF value ranging around 2-3 [4]. This shows that YM strain has similar SPF value as marine microbes which constantly undergoes environmental stress. The SPF value Nano emulsion of bruiti oil and microbial carotenoids was estimated up to 36 suggesting the room for improvement of microbial carotenoids as High SPF using natural compounds [38].

Table 1: Level of variables used in Central Composite Design for optimization of pigment production by *Rhodotorula mucilaginosa* YM

Variables	Units	-alpha	Low	High	+alpha
pH (A)	pH	2	3.5	7.5	8.8
Inoculum density (B)	% (v v^{-1})	0.3	1	3	3.6
Incubation Time (C)	h	7.6	24	72	88.3

Table 2: Experimental design CCD used to optimize the parameters for carotenoid production of *Rhodotorula mucilaginosa* YM

	pH	Inoculum density (%v v^{-1})	Incubation Time (h)	Total Carotenoids* (mg L^{-1})
1	3.5	3	24	26.5±0.4
2	7.5	3	72	29.5±0.8
3	5.5	2	48	51.32±0.6
4	7.5	1	72	24.41±0.2
5	5.5	2	48	49.8±0.7
6	7.5	1	24	12.45±0.2
7	5.5	2	48	46.17±0.7
8	3.5	3	72	29.85±0.8
9	5.5	2	48	51.32±0.6
10	7.5	3	24	23.28±0.2
11	3.5	1	72	25.93±0.3
12	5.5	0.3	48	18.72±0.1
13	5.5	2	48	51.32±0.6
14	2.1	2	48	10.93±0.0
15	5.5	2	88.3	32.74±0.2
16	5.5	3.6	48	33.43±0.4
17	3.5	1	24	15.04±0.1
18	5.5	2	48	51.32±0.6
19	8.8	2	48	8.87±0.3
20	5.5	2	7.6	19.8±0.2

Note: *All factorial and axial points are means of duplicates

Table 3: Analysis of variance (ANOVA) for Quadratic model of carotenoid production

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3520.06	9	391.12	42.75	< 0.0001	significant
A-pH	9.09	1	9.09	0.9941	0.3423	
B-Inoculum density	229.95	1	229.95	25.13	0.0005	
C-Incubation Time	214.96	1	214.96	23.50	0.0007	
AB	0.0364	1	0.0364	0.0040	0.9509	
AC	1.94	1	1.94	0.2121	0.6550	
BC	22.04	1	22.04	2.41	0.1516	
A^2	2240.79	1	2240.79	244.93	< 0.0001	
B^2	656.76	1	656.76	71.79	< 0.0001	
C^2	643.42	1	643.42	70.33	< 0.0001	
Residual	91.49	10	9.15			
Lack of Fit	59.53	5	11.91	1.86	0.2556	not signifi-cant
Pure Error	31.96	5	6.39			
Cor Total	3611.55	19				

Table 4: Statistical summary of the Central Composite model used in Carotenoid production

Terms	Total Carotenoid Production
F- value	42.75
P > F*	0.0001
Mean	29.89
C.V%	10.12
R2	0.9747
Adjusted R^2	0.9519
Predicted R^2	0.8627
Adequate precision	17.3038

Note: *Prob > F less than 0.05 indicates that the model terms is significant.

Table 5: Determination of Sun Protection Factor (SPF) for carotenoid by UV Spectrophotometry

S. No.	Wavelength [λ](nm)	EE X I (normalized)	Absorbance Value[abs(λ)]	EE(λ) × I(λ) × abs(λ)
1	290	0.0150	0.387	0.0058
2	295	0.0817	0.358	0.0292
3	300	0.2874	0.336	0.0966
4	305	0.3278	0.313	0.1026
5	310	0.1864	0.284	0.0529
6	315	0.0837	0.269	0.0225
7	320	0.0180	0.232	0.0042
TOTAL ($\sum^{320}$ **EE**(λ) × **I**(λ) × **abs**(λ)) 290				0.3138
SUN PROTECTION FACTOR (CV × TOTAL)				3.138

Abbreviations: CV, correction value; EE, spectrum of erythema effect; I, spectrum of sun's intensity; Abs, absorbance of sample.

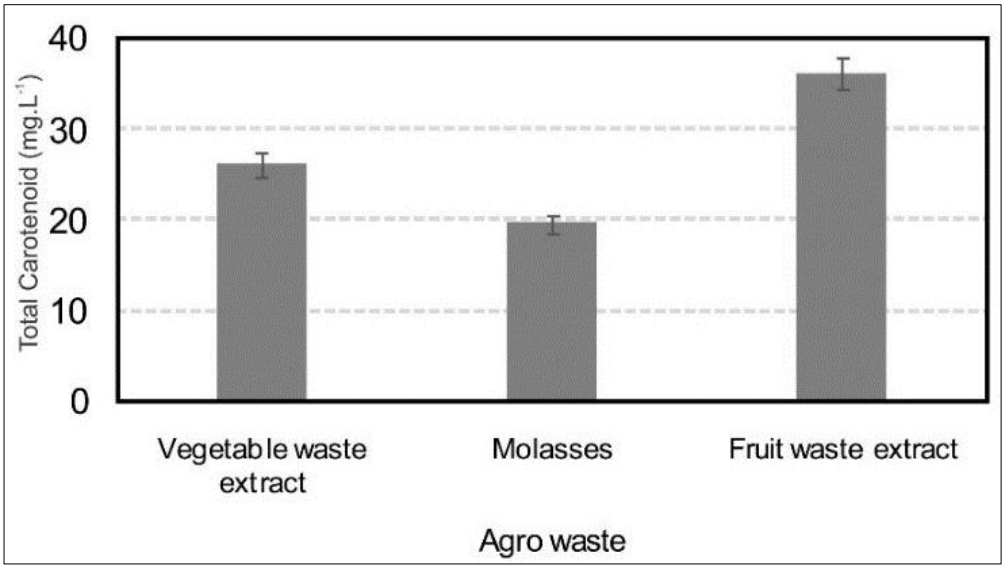

Fig 1: Production of Carotenoid using agro waste by *Rhodotorula mucilaginosa* YM

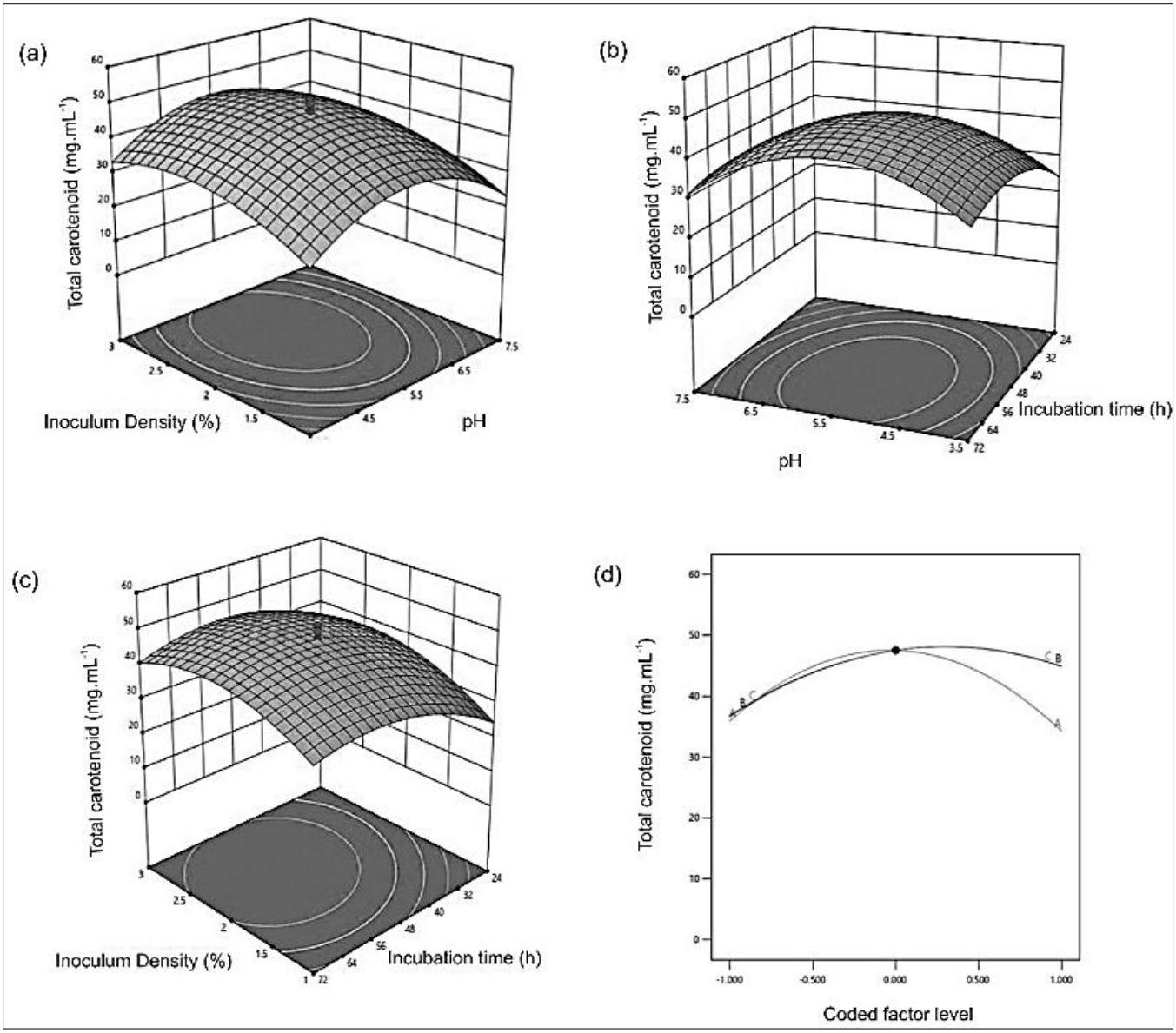

Fig 2: Three dimensional plots showing interaction between (a) Inoculum density (%) and pH, (b) pH and Incubation Time (h) and (c) Inoculum density (%) and Incubation Time (h). (d) Perturbation curve of the model for carotenoid production by *Rhodotorula mucilaginosa* YM

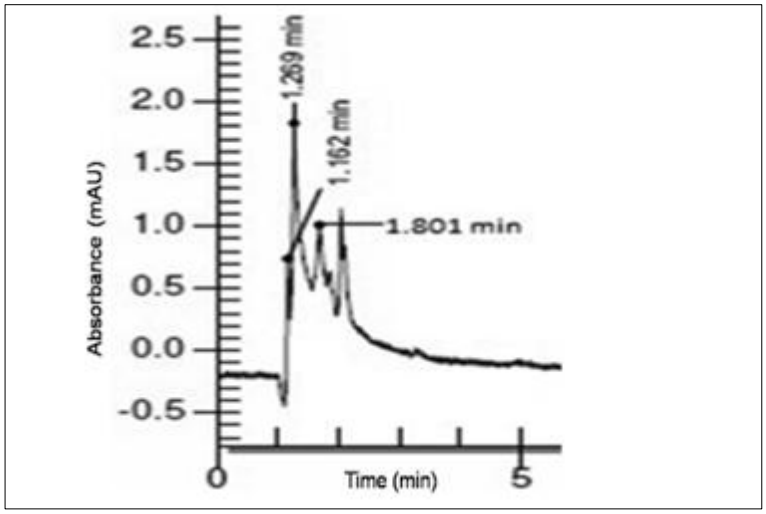

Fig 3: High-performance liquid chromatography chromatogram of carotenoid extracted using silica column and 90% methanol

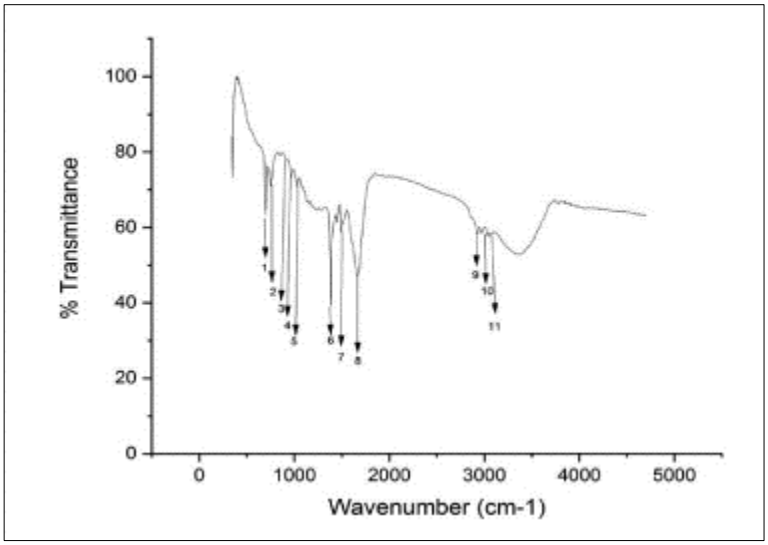

Fig 4: Spectrum of FT-IR analysis for carotenoid extract from *Rhodotorula mucilaginosa* YM

Conclusions

From this present study it can be concluded that Fruit Waste Extract appears to be a promising substrate since there is a remarkable carotenoid production without any other substrate supplements and special conditions. The Central Composite Design was proved to be effective for optimizing the fermentation conditions for commercially important carotenoid compounds like Astaxanthin by *R. mucilaginosa* YM with Fruit Waste substrate. After optimization maximum yield was at pH 5.5, inoculum volume 2% (v v^{-1}) and with reduction in incubation time to 48 h. The Sun Protection Factor of carotenoid and coloring properties encourages its applicability as a constitutional ingredient in cosmetics and pharmaceuticals. It is fervently found that the yeast carotenoid pigment can be produced reutilizing fruit market wastes could receive greater attention. Thus, carotenoids extracted employing *R. mucilaginosa* YM have great potential for various applications, with implications for the sustainable and cost-effective industrial production.

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References

1. Maoka T. Carotenoids as natural functional pigments. J Nat Med. 2019 Oct;74. https://doi.org/10.1007/s11418-019-01364-x
2. Ren Y, Sun H, Deng J, Huang J, Chen F. Carotenoid Production from Microalgae: Biosynthesis, Salinity Responses and Novel Biotechnologies. Mar Drugs. 2021 Dec;19:713. https://doi.org/10.3390/md19120713
3. Maj A, Dziewit L, Drewniak L, Garstka M, Krucon T, Piatkowska K, *et al*. *In vivo* creation of plasmid pCRT01 and its use for the construction of carotenoid producing *Paracoccus* spp. strains that grow efficiently on industrial wastes. Microb Cell Factories. 2021 Jul;19:141. https://doi.org/10.1186/s12934-020-01396-z
4. Kusmita L, Nuryadi H, Widyananto PA, Muchlissin S, Sabdono A, Trianto A, *et al*. Bioactivity of carotenoid produced by soft coral symbiotic microorganisms from Panjang and Karimunjawa Island, Central Java, Indonesia. J Biol Divers. 2021 Feb;22(2):732-740. https://doi.org/10.13057/biodiv/d220226
5. Poddar K, Padhan B, Sarkar D, Sarkar A. Purification and optimization of pink pigment produced by newly isolated bacterial strain *Enterobacter* sp. PWN1. SN Appl Sci. 2021 Jan;105(3). https://DOI.org/10.1007/s42452-021-04146-x

6. Rao MPN, Xiao M, Li WJ. Fungal and bacterial pigments: Secondary metabolites with wide applications. Front Microbiol. 2017 Jun;22(8):1113. https://DOI.org/3389/fmicb. 2017.01113
7. Adadi P, Barakova NV, Krivoshapkina EF. Selected methods of extracting carotenoids, characterization, and health concerns: A review. J Agric Food Chem. 2018 May;66(24):5925-5947. https://DOI.org/10.1021/acs.jafc.8b01407
8. Saini RK, Keum YS. Progress in Microbial Carotenoids Production. Indian J Microbiol. 2017 Jan;57:129-130. https://dx.DOI.org/10.1007%2Fs12088-016-0637-x
9. Wang C, Zhao S, Shao X, Park JB, Jeong SH, Park HJ, *et al*. Challenges and tackles in metabolic engineering for microbial production of carotenoids. Microb Cell Factories. 2019 Mar;55(18). https://DOI.org/10.1186/s12934-019-1105-1
10. Sinha S, Singh G, Arora A, Paul D. Carotenoid Production by Red Yeast isolates grown in Agricultural and "Mandi" Waste. Waste and Biomass Valori. 2021 Jul;12(7):1-11. https://DOI.org/10.1007/s12649-020-01288-8
11. Cipolatti EP, Remedi RD, Sa CDS, Rodrigues AB, Ramos JMG, Burkert CAV, *et al*. Use of agro-industrial byproducts as substrate for production of carotenoids with antioxidant potential by wild yeasts. Biocatal Agric Biotechnol. 2019 Jul;20:101208. https://doi.org/10.1016/j.bcab.2019.101208
12. Kaur P, Ghoshal G, Jain A. (2018) Bio-utilization of Fruits and Vegetables Waste to Produce Carotene in Solid-State Fermentation: Characterization and antioxidant activity. Process Biochem. 2018 Jan;76:155-164. https://doi.org/10.1016/j.procbio.2018.10.007
13. Abdelhafez AA, Husseiny SM, Ali AAA, Sanad HM. Optimization of β- carotene production from agro-industrial by-products by *Serratia marcescens* ATCC 27117 using Plackett-Burman design and central composite design. Ann Agric Sci. 2016 Jun;61(1):87-96. https://doi.org/10.1016/j.aoas.2016.01.005
14. Sen T, Barrow CJ, Deshmukh SK. Microbial pigments in the food industry- challenges and the way forward. Front Nutr. 2019 Mar, 6. https://doi.org/10.3389/fnut.2019.00007
15. Machado WRC, Silva LGD, Vanzela ESL, Del Bianchi VL. Evaluation of the process conditions for the production of microbial carotenoids by the recently isolated *Rhodotorula mucilaginosa* URM 7409. Braz J Food Technol. 2019 Jan;22(2). http://dx.DOI.org/10.1590/1981-6723.26718
16. Vega RIG, Lopez JLC, Elias JAL, Cruz SR, Diaz AR, Perez LMP, *et al*. Optimization of growing conditions for pigments production from microalga *Navicula incerta* using response surface methodology and its antioxidant capacity. Saudi J Biol Sci. 2021Feb;28(2):1401-1416. https://DOI.org/10.1016/j.sjbs.2020.11.076
17. Nowak KW, Mielnik P, Sięda M, Staniszewska I, Bieniek A. The effect of ultrasound treatment on the extraction of lycopene and β-carotene from cherry silverberry fruits. AIMS Agric. Food. 2021 Jan;6(1):247-254. https://DOI.org/10.3934/agrfood.2021016
18. Lobo PTD, Brandao HN, de Assis SP. Production of carotenoids by *Rhodotorula mucilaginosa* using sugarcane juice (*Saccharum officinarum*) in the fermentation. Braz J Dev. 2021 Jan;7(2):18235-18250. http://dx.doi.org/10.34117/bjdv7n2-455
19. Garuba EO, Ajunwa OM, Olaifa KW, Onilude AA. Response Surface Methodology (RSM) and electromagnetic optimization of pigment production by *Sporobolomyces* sp S5 and *Rhodotorula* sp A21 in submerged fermentation. J Biosci Biotechnol. 2020 Jun;9(1):17-25.
20. Garcia-Cortes A, Garcia-Vasquez, JA, Aranguren Y, Ramirez-Castrillon, M. Pigment Production Improvement in *Rhodotorula mucilaginosa* AJB01 Using Design of Experiments. Microorganisms. 2021 Feb;9(2):387. https://DOI.org/10.3390/microorganisms9020387
21. Khattab SMR, Hadi AMA, Dahab NFA, Atta OM. Isolation, Characterization, and Identification of Yeasts Associated with Foods from Assiut City, Egypt. Br Microbiol Res J. 2016 Feb;13:1-10. http://dx.DOI.org/10.9734/BMRJ/2016/24170
22. Asker D, Awad TS, Beppu T, Ueda K. Isolation, Characterization, and Diversity of Novel Radiotolerant Carotenoid-Producing Bacteria. In: Barredo JL. (eds) Microbial Carotenoids from Bacteria and Microalgae. Methods in Molecular Biology (Methods and Protocols), Humana Press, Totowa, NJ, 2012, 21-60pp. http://DOI.org/10.1007/978-1-61779-879-5_3
23. Costa WA, Padilha CEA, Junior SDO, Silva FLH, Silva J, Ancantara MA, *et al*. Oil-lipids, carotenoids and fatty acids simultaneous production by *Rhodotorula mucilaginosa* CCT3892 using sugarcane molasses as carbon source. Brazilian J Food Technol, 2020 Apr;23. https://DOI.org/10.1590/1981-6723.06419
24. Kanzy HM, Nasr NF, Shazly HAE, Barakat OS. Optimization of carotenoids production by yeast strains of *Rhodotorula* using salted cheese whey. Int Curr Microbiol Appl Sci. 2015 Jan;4(1):456-469.
25. Saejung C, Puensungnern L. Evaluation of Molasses-Based Medium as a Low Cost Medium for Carotenoids and Fatty Acid Production by Photosynthetic Bacteria. Waste Biomass Valori, 2018 Jan, 11. https://doi.org/10.1007/s12649-018-0379-6
26. Korumilli T, Mishra S, Korukonda JR. Production of astaxanthin by *Xanthophyllomyces dendrorhous* on fruit waste extract and optimization of key parameters using Taguchi method. J Biochem Technol. 2020 Aug;11(1):25-31.
27. Mao X, Sun R, Tian Y, Wang D, Ma Y, Wang Q, *et al*. Development of Solid Self-Emulsification Delivery System for the Oral Delivery of Astaxanthin. Eur J Lipid Sci Technol. 2019 Mar;121(5):1800258. https://doi.org/10.1002/ejlt.201800258
28. Sayre RM, Agin PP, Levee GJ, Marlowe EA. Comparison of *in vivo* and *in vitro* testing of sun screening formulas. Photochem Photobiol. 1979 Mar;29:559-566. https://DOI.org/10.1111/j.1751-1097.1979.tb07090.x
29. Mbanga L, Mulenga M, Mpiana PT, Bokolo K, Mumbwa M, Mvingu K. Determination of Sun Protection Factor (SPF) of some body creams and lotions marketed in Kinshasa by Ultraviolet

Spectrophotometry. Int J Adv Res Chem Sci. 2014 Oct;1(8):7-13.
30. Rodrigues TVD, Amore TD, Teixeira EC, De Medeiros Burkert JF. Carotenoid Production by *Rhodotorula mucilaginosa* in Batch and Fed-Batch Fermentation Using Agroindustrial Byproducts. Food Technol Biotechnol, 2019 Jun;57(3). https://DOI.org/10.17113/ftb.57.03.19.6068
31. Leyton A, Flores L, Arvela PM, Lienqueo ME, Shene C. *Macrocystis pyrifera* source of nutrients for the production of carotenoids by a marine yeast *Rhodotrula mucilaginosa*. J Appl Microbiol. 2019 Oct;127:1069-1079. https://DOI.org/10.1111/jam.14362
32. Tarangini K, Mishra S. Carotenoid production by *Rhodotorula* sp. on fruit waste extract as a sole carbon source and optimization of key parameters. Iran J Chem Chem Eng. 2014 Oct;33:89-99. https://dx.DOI.org/10.30492/ijcce.2014.11344
33. Chelladurai SJS, Murugan K, Ray AP, Upadhyaya M, Narasimharaj V, Gnanasekaran S. Optimization of process parameters using response surface methodology: a review. Mater Today Proceedings. 2021;37:1301-1304. https://DOI.org/10.1016/j.matpr.2020.06.466
34. Zhao Y, Guo L, Xia Y, Zhuang X, Chu W. Isolation, identification of carotenoid- producing *Rhodotorula* sp. from marine environment and optimization for carotenoid production. Mar Drugs. 2019 Mar;17(3):161. https://dx.DOI.org/10.3390%2Fmd17030161
35. Sharma R, Ghoshal G. Optimization of Carotenoids production by *Rhodotorula mucilaginosa* (MTCC-1403) using agro-industrial waste in Bioreactor: A statistical approach. Biotechnol Rep. 2020 Mar;e00407. http://DOI.10.1016/j.btre.2019.e00407
36. Chen X, Chen R, Guo Z, Li C, Li P. The preparation and stability of the inclusion complex of astaxanthin with β-cyclodextrin. Food Chem. 2007;101(4):1580-1584. https://DOI.org/10.1016/j.foodchem.2006.04.020
37. Davinelli S, Nielsen ME, Scapagnini G. Astaxanthin in skin health, repair, and disease: A comprehensive review. 2018 Apr;10(4):522. Nutr https://dx.DOI.org/10.3390%2Fnu10040522
38. Mansur MCPPR, Campo C, Vermelho AB, Nobrega J, Boldrini LDC, Balottin L, *et al*. Photoprotective nanoemulsions containing microbial carotenoids and buriti oil: Efficacy and safety study. Arab J Chem. 2020 Aug; 13:6741-6752. https://DOI.org/10.1016/j.arabjc.2020.06.028