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Research Article

Stability analysis of nano-dispersed food bio-colour extracted from coconut milk as a substrate by using yeast (*Xanthophyllomyces dendrorus*)

V. Monishdeep, H. W. Despande, A. Poshadri

Abstract

Food color plays a prominent role in the sensory evaluation of any food materials; because of this it affects the uniqueness, desirability, and palatability of food. Properties such as easy synthesis and cost-effectiveness made synthetic colors used more in different kinds of food. Many synthetic dyes have been causing several adverse effects and toxic diseases including Attention Deficit Hyperactivity Disorder (ADHD), hyper-allergenicity, food allergies, depression, headaches, carcinogenicity, migraines, and other toxicological issues like issues related to children's behavior. This has been a driving force for many food technologists all over the world to show interest in replacing synthetic colors with natural colorants as per the market demand as well as clean-label product promotion. Biocolors are not only color foods but also have medical and therapeutic effects. They are derived from natural sources including plants, animals, algae, fungi, and insects that can impart certain colors to foods. Microorganisms are classified as a highly significant source for biocolour production due to their merits like durability, availability, labor, cost-effectiveness, productivity, and simple downstream processing in comparison with plants. Coconut milk was used as the substrate for biocolour preparation by using yeast(*Xanthophyllomyces dendrorus*), where maximum biomass yield was 5.15±0.096 g/L, extracted colour (astaxanthin) yield of 0.84±0.024 g/L and maximum nano-dispersed color yield 0.83±0.024g shows efficiency more than 90% was produced. The Nano-dispersed color incubated under conditions including varied light intensities or conditions, different pH, and different temperatures were observed with degradation of biocolour with $p<0.01$ high significance.

Keywords astaxanthin, biocolour, carotenoids, *xanthophyllomyces dendrorus*

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Introduction

Food color plays a prominent role in the sensory evaluation of any food materials, because of this it affects the uniqueness, desirability, and appetizingness of food [1]. The coloring of food is not new to mankind; color is added to food to make up for the loss of color during the process followed by an increase in the quality and appealingness of food. Many food quality parameters like freshness and flavor are hooked with color of food, thus it is used as a tool in sensory evaluation. Properties like easy synthesis and cost-effectiveness made synthetic colors used more in different kinds of food [2]. Various artificial colorants most frequently

employed in many food products, pharmaceuticals, cosmetics, and dyes have shown many adverse effects and toxic diseases including Attention Deficit Hyperactivity Disorder (ADHD), hyper-allergenicity, food allergies, depression, headaches, carcinogenicity, migraines, and other toxicological issues like issues related to children behavior. Many toxicological studies of artificial colorants revealed various ad-verses that made them to eliminate or completely remove from the list of authorized food colors [3]. This made many food technologists all over the world to show interest in replacing synthetic colors to replace completely by developing natural colorants as per many consumer's demand [4]. Biocolors are pigment or dyes, when used in foods along with colouring them, also gives medical and therapeutic effects. They are components that can colour food that are derived from natural sources including plants, animals, algae, fungi, and insects that can impart certain colour to foods. Most are plant derivatives due to their renewability. The use of biocolors for colouring food is less certain because of their preferability as medicinal agents [3]. The global market size of natural colorants is 600 million dollars and annually growing at the rate of 7% which accounts for a sale of 29% [1]. The natural dyes can be sourced from the following sources mentioned below.

1. Plants (Fruits, Flowers, roots, seeds, etc.)
2. Animals (Lac, Cochineal, etc.)
3. Microorganisms

Microorganisms are classified as a highly significant source for biocolour production due to their merits like durability, availability, labour, cost-effectiveness, productivity, and simple downstream processing in comparison with plants. Many biocolors like flavins, carotenoids, violancein, melanins, monascins, and quinines, can be produced by using microbes. Organic waste from agro-processing industries can serve as a substrate for solid and submerged state fermentation by microbes. Numerous biocolors are used in the food and cosmetics industries along with the property of colouring them they also possess some properties like anti-inflammatory, anticancer, antioxidant, and anti-microbial properties [5].

Carotenoids are a highly interesting and explored pigment class among all bio-pigments by researchers. They are components with high sensitivity for light, heat, and oxygen along with lipid solubility and show some association with fractions of lipid fractions. They show the most prominent role in the protection and prevention of oxidative damage. They are capable of boosting the immune system and preventing of many malignancies [6]. Astaxanthin which is known as 3, 3-dihydroxy-ß, β 0-carotene-4, 4-dione according to IUPAC is a keto carotenoid imparts orange-pink colour which can be synthesized by the addition of Hydroxyl and keto-moieties to ß-carotene [7]. Microalgae (*Haematococcus pluvialis*) and yeast (*Xanthophyllomyces dendrorhous*) are the most prominent developers of astaxanthin in microorganisms and are the most possible carotenoid bio-sources. They are utilized in dairy products, beverages, and meats for commercial colouring. Thus, it is present in red yeast (*Xanthophyllomyces dendrorus*) as a primary carotenoid and which can be used as colorant in food. Currently, Microalga (*Haematococcus pluvialis*) and yeast (*Xanthophyllomyces dendrorhous*) are used for the extensive growing of astaxanthin. *Agrobacterium aurantiacum*, *Paracoccus carotinifaciens,* and *Paracoccus marcusii*, are a few bacteria's' that can produce astaxanthin [8].

Colour (astaxanthin) helps in the promotion of many important biological processes including protection against UV rays and boosts immunological response, along with the significant antioxidant activity. Food, animal feeding, cosmetic, and pharmaceutical industries all rely on astaxanthin for the nutrition of animals, especially in a sector like aquaculture. *Xanthophyllomyces dendrorhous* (previously *Phaffia rhodozyma*) is a potential alternative for commercial astaxanthin production because of its characteristic reliability as a carotenoid source. The production of astaxanthin by *X. dendrorhous* in lab scale is to maximize synthesis of astaxanthin by analyzing the impact of operational circumstances or carbon sources and other elements [7-8]. Agro-waste that can be used as the substrate for colour (astaxanthin) preparation by *Xanthophyllomyces dendrorus* includes molasses [9], cellobiose [10-11], peat hydrolysates [12], Aqua search Growth Module[13], date juice from *Yucca fillifera* [14-15], coconut-milk [16], Sweet sorghum [17], yeast peptone dextrose and

yeast mold [18], cassava starch [11], pineapple[19], distiller's dried grain soluble [20], and biomass made of lignocellulose and stillage [21]. Astaxanthin is incredibly vulnerable to oxidation, thermal treatments, and light because of its high unsaturation. it is extremely water-insoluble to overcome these demirt unique mechanisms like micro or nano delivery mechanisms including microencapsulation technique [22], astaxanthin emulsions [23], astaxanthin oleoresin emulsion [24], nanostructured lipid carriers [25] microencapsulates of astaxanthin [26], and multilayer emulsions' encapsulation [27] are in use, especially Nano-dispersion systems are prominently essential. Scattered and fine nanoparticles make up Nano-dispersions in persistent aqueous solutions. Nano-dispersion technology is best suited for shrinking substances at Nano scale which also enhances saturation solubility, dissolving rate, and biological usefulness. Hence, it is possible to make the surface of bioactive Nano-sized components in the dispersal system, especially as a convenient tool for carrying components and making them water soluble. Because of the extremely small particle size and large surface area of Nano dispersions possess potency to enable lipophilic active chemicals which are rarely water soluble [28-29].

Methodology

Growth media

Colour (astaxanthin) was prepared by the method in Dominguez-Bocanegra et al., [16] work by utilizing Coconut milk as the sole source. The coconut milk was autoclaved (121°C and 15psi for 30 min) to prepare to sterilize the media. And it was inoculated with an inoculum of 10% i.e, culture freeze-dried *Xanthophyllomyces dendrorus* (NCIM number 3644 and ATCC number 24202 was collected from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune India) which was made active or revived with Yeast-malt broth (0.3g Malt extract, 1.0g glucose, 0.3g yeast extract, 0.5g peptone, 100 ml distilled water) and incubated in an orbital shaking incubator at 25°C with 150 rpm till colour changes to pink which took 5 days.

Extraction of colour

The colour prepared was crude with impurities and was harvested by the combination of physical cell disruption followed by chemical extraction technique as the method in Dominguez-Bocanegra et al., [16]. 1ml Aliquot was triplicated in three Erlenmeyer flasks with saturated culture and was centrifuged at 5000 rpm for 5 min. The cell pellets were washed twice with 1 ml. deionized water and 1 ml acetone till complete evaporation of solvent takes place.1ml glass pearls (425-600 µm diam) and 1ml. Dimethylsufoxide (DMSO) at 60°C was added followed by vigorous shaking to extract colour, the organic phase was vortex for 5 min. Subsequently, 1 ml acetone, 1 ml NaCl at 20% w/v and 1 ml petroleum ether were also added. Finally, colour from petroleum ether was subjected to centrifugation at 5000 rpm for 5 min. Spectrophotometer (474 nm) was used to quantify the carotenoid.

Nano-dispersion of biocolour

Water insolubility is one prominent demerit that made the biocolour (astaxanthin) to use in very limited foods as the colorant. To counter act the demerit various techniques are in use and Nano-dispersion technology best a suited technique. Nano-dispersion of colour (astaxanthin) was prepared with the method of Anarjan and Tan [28-29]. 2.5% w/w Stabilizer (polysorbate-80 and gaur gum) and 0.02 % sodium azide were added to 0.05M phosphate buffer (pH 7) at 40°C and stirred overnight with a magnetic stirrer followed by centrifugation in the next morning at 3000 rpm for 5min., 68% w/w acetone and 32 % w/w dichloromethane and 0.08% w/w colour (astaxanthin) were mixed. The 11.5% colour mixture was added to the stabilizer mixture and homogenized using a conventional homogenizer at 5000 rpm for 5 min. Additionally, the solution containing colour and stabilizers mixture was passed thrice through a high-pressure homogenizer at 30 MPa which forms

nano-emulsion. The Nano-emulsion was subjected to evaporation to remove all solvents employing a rotary evaporator with 100 rpm and 150 Pa at 25°C so that Nano-dispersion of colour (astaxanthin) was formed.

Colour stability of Nano-dispersion of colour (astaxanthin)

Nano-dispersion of colour (astaxanthin) was analyzed for stability with the method of Mhalaskar et al., [30]. The analysis of colour stability was done with 1000 ppm (10 ml each) Nano-dispersed colour solution in test tubes and were incubated at varied conditions. Nano-dispersed colour solution was wrapped with aluminum foil and incubated under dark, fluorescent and UV light for light stability analysis for 7 days. For thermal stability analysis, Nano-dispersed colour solutions in tubes were wrapped with aluminum foil and kept for incubation at temperatures i.e, 4, 10, and 25°C for 25 days. The same procedure was followed for pH stability analysis and pH was adjusted for neutral (pH=7.0), basic (pH=9.5) and acidic (pH=3.0), with the addition of either 0.1 N NaOH for basicity and 0.1 N HCl for acidity and incubated for 7 days. The Optical Density of the original blank sample along with all incubated samples was measured and compared. The Optical density of samples at the 2nd hour, 4th hour, 6th hour, and 8th hour after incubation of day one and followed by a reading every day till day 7 for light and pH effect analysis, whereas for temperature effect till day 25 by employing Spectrophotometry at 475 nm. The results are expressed as the percent initial absorbance and retained percent of colour after subjection to varied treatments and the initial absorbance percent retained at any time give the stability of the particular samples.

Results and Discussion

The main objective of this research work was to prepare biocolour using yeast culture *Xanthophyllomyces dendrorus* from the substrate of coconut milk. *X. dendrorus* is known organism, which produces a prominent pigment called astaxanthin. Astaxanthin is a carotenoid pigment that imparts pink colour to foods when added in small quantities. It is having functional benefits such as antioxidant, anti-carcinogenic, anti-inflammatory, anti-aging, etc. The extracted biocolour astaxanthin is converted into nano-dispersed astaxanthin to replace synthetic food colour in different foods. Sincere efforts have been taken to evaluate it stability of nano-dispersed astaxanthin colour solution.

Yield of astaxanthin

Coconut milk was used as a source of carbon source and minerals it can serve as a sole source for the production of color (astaxanthin) with the yeast *Xanthophyllomyces dendrorus*. After fermentation on the 5th day, the maximum yield of biomass was 5.15±0.096 g/L and astaxanthin quantified after chemical extraction from yeast fermented biomass was an average of 0.84±0.024 g/L.

Yield of Nano-dispersed Astaxanthin

The colouring pigment astaxanthin is lipid soluble and insoluble in water. However, there is a need to explore its functional health benefits in all kinds of foods with its addition. The fat-soluble astaxanthin was converted into the nano-dispersion of astaxanthin to be compatible with all kinds of food. The yield obtained after nanodispersion of color (astaxanthin) was 0.83±0.024g and recorded 90% efficiency during conversion.

Stability of Nano-dispersed color

Stability under different light conditions

Degradation of carotenoid may also depends on light to which it is exposed. Radical-cat-ions are resultants when carotenoids are exposed to photo-oxidation. Light exposure may excite the molecule when they come to a lower excitation state and may react with molecules (radical by products) in the

media by abstraction of hydrogen and form carotenoid radical cat-ions possibly [29]. Since the color prepared is carotenoid so to analyze the stability of color [28], it was subjected to different light conditions and percentage degradation was calculated. Color (Nano-dispersed astaxanthin) incubated under Ultra-violet light took 5 days to degrade >95% and color incubated under fluorescent light took 6 days to degrade >85%. Whereas, color incubated under dark conditions retained >80% of color even after 7th day with $p<0.01$ highly significant difference of all light conditions same is interpreted in Table 1 and sketched in Figure 1.

Table 1. Percentage retention of color at a particular period under light

Time	UV	Fluorescent	Dark
Initial	100±1.692	100±1.692	100±1.364
2hr	98.834±1.692	98.542±1.991	99.708±1.36
4hr	95.802±1.62	97.260±1.965	99.009±1.351
8hr	92.451±1.564	95.657±1.933	97.114±1.325
2day	45.671±0.733	81.958±1.656	96.531±1.317
3day	3.847±0.065	68.988±1.394	94.637±1.291
4day	1.573±0.027	53.803±1.087	91.693±1.251
5day	0.174±0.003	35.324±0.714	87.467±1.193
6day	0±0	20.751±0.419	85.193±1.162
7day	0±0	8.423±0.17	82.279±1.123
C.D.	3.162**	4.429**	3.792**
S.E (m)	1.064	1.491	1.276
C.V.%	4.199	3.87	2.329

** is Highly-significant, * is significant

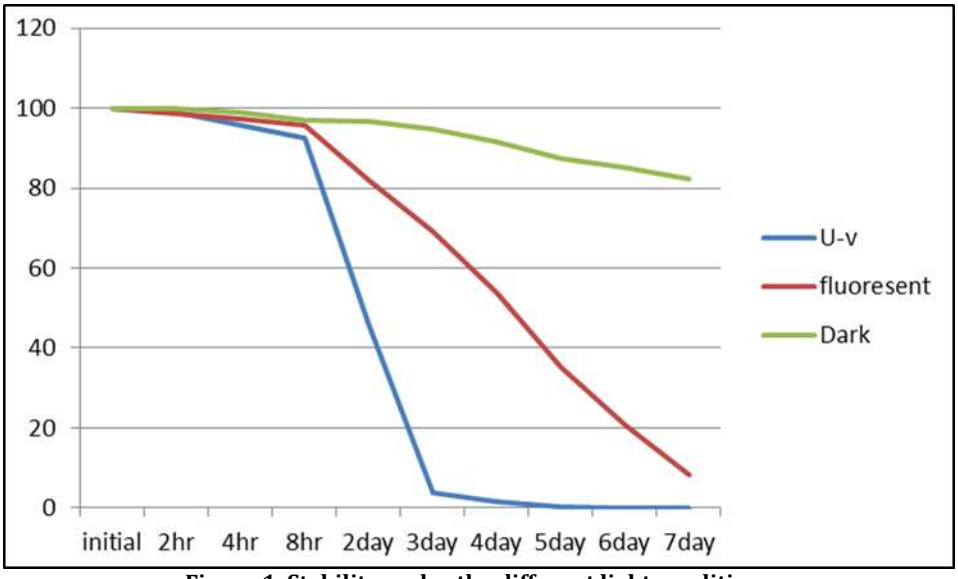

Figure 1. Stability under the different light conditions

Stability under different pH range

pH is the potential of hydrogen is a scale used to specify the acidity or basicity of the aqueous solution. pH of the food is an important property to promote or deny the preservation of any food thus it is a very important property to be considered for stability. Color (Nano dispersed astaxanthin) is a carotenoid and pH affects the degradation of carotenoids i.e., at lower pH or acidity, the stability of carotenoid is prominently more compared to higher pH or basicity and neutral pH. The according

to data obtained degradation was observed in all three pH ranges but the degradation of color is less in acidic pH than in neutral and basic pH with $p<0.01$ highly significant difference and the same is represented in Table 2 and shown in Figure 2.

Table 2. Percentage retention of color at a particular period under different pH

Time	Acid	Neutral	Basic
Initial	100±1.692	100±1.692	100±1.692
2hr	98.498±1.666	98.582±1.992	98.667±1.346
4hr	97.178±1.644	93.968±1.899	94.368±1.287
8hr	95.526±1.616	92.400±1.867	92.794±1.266
2day	81.416±1.377	84.378±1.705	76.627±1.046
3day	68.057±1.151	62.2431.258	62.579±0.854
4day	52.416±0.887	46.531±0.94	46.684±0.637
5day	33.383±0.565	28.076±0.567	30.971±0.422
6day	18.372±0.311	14.445±0.292	15.501±0.211
7day	5.673±0.096	2.653±0.054	2.633±0.036
C.D.	3.685**		2.878**
S.E (m)	1.24		0.969
C.V.%	3.297		2.658

** is Highly-significant, * is significant

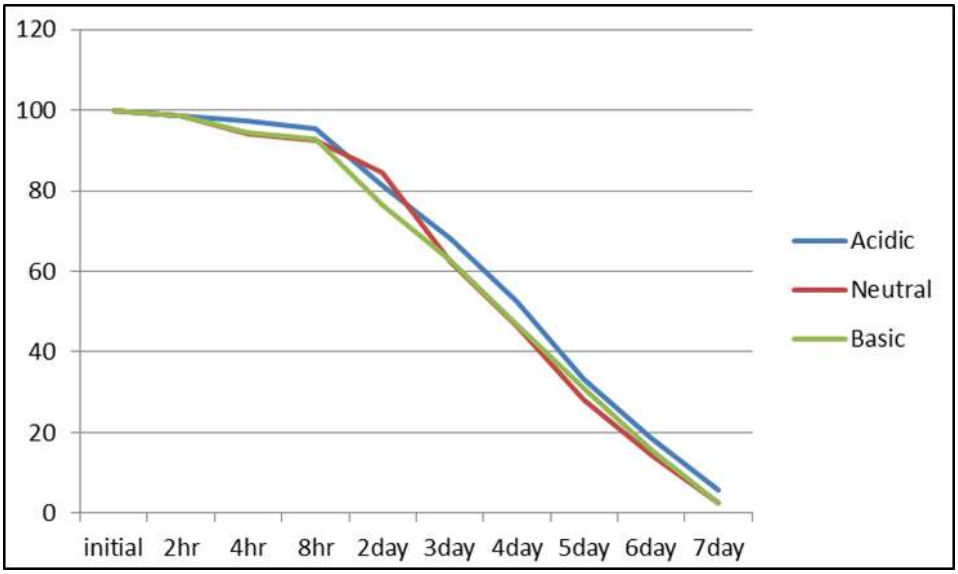

Figure 2. Stability under the different pH range

Stability under different temperature range

Temperature is an important parameter for almost all activities done for the preservation and processing of food. Temperature plays a very prominent role in many reactions and lower temperature may impair many reactions and ambient temperature promotes many reactions. To analyze the color stability to temperature it is incubated under different temperatures i.e., 4°C, 10°C, and 25°C. The color incubated under 25°C took 20 days to degrade > 95% followed by color incubated at 10°C took more than 25 days to degrade 75% and whereas color incubated at 4°C retained >50% color even after 25th day of incubation with $p<0.01$ highly significant difference between all three temperature range and same is in Table 3 and represented in Figure 3.

Table 3. Percentage retention of color at a particular period under different storage temperatures

Time	25°C	10°C	4°C
initial	100±1.692	100±1.692	100±1.692
2hr	98.775±1.671	98.484±1.99	99.242±1.354
4hr	95.890±1.622	97.318±1.967	98.251±1.34
8hr	95.890±1.622	97.318±1.957	98.251±1.34
5day	67.269±1.138	81.871±1.554	87.846±1.198
10day	45.788±0.774	69.105±1.397	80.763±1.102
15day	21.538±0.364	53.949±1.09	71.174±0.971
20 day	2.593±0.044	38.618±0.78	62.139±0.848
25 day	0±0	23.666±0.478	53.561±1.14
C.D.	3.591**	4.733**	3.585**
S.E(m)	1.199	1.581	1.197
C.V.%	3.536	3.695	2.35

** is Highly-significant, * is significant

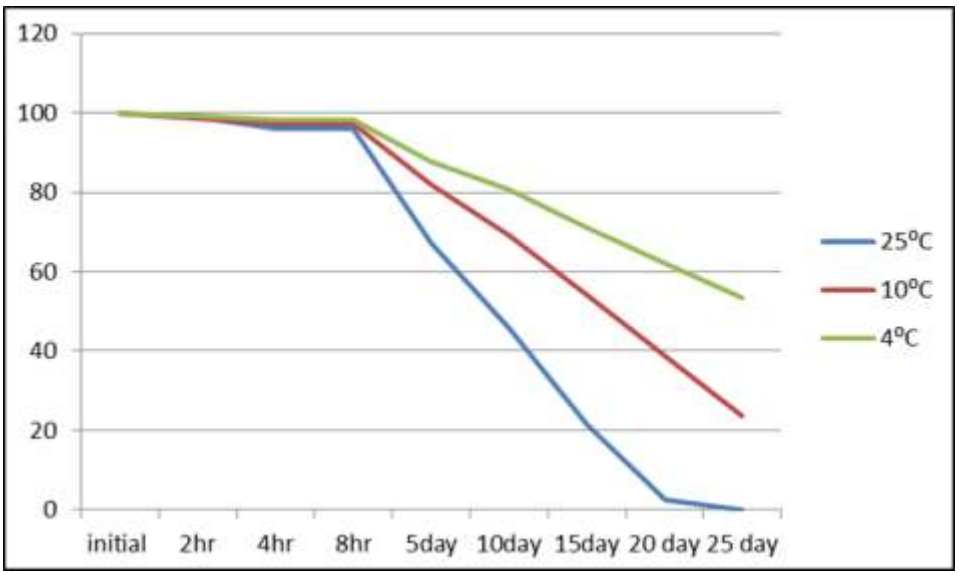

Figure 3. Stability under the different Temperature range

Conclusion

The food color is one of the important ingredients on which many quality parameters are dependent so before using any color in food, its safety and stability should be predominant parameters under consideration. Astaxanthin is a carotenoid and pH affects the degradation of carotenoids. The degradation of color is less in acidic pH than in neutral and basic pH ($p<0.01$). Nano dispersed astaxanthin solution was incubated under Ultra-violet light and took 5 days to degrade >95% and color incubated under fluorescent light took 6 days to degrade >85%, whereas color incubated under dark conditions retained >80% of color even after 7th day. It was observed that biocolour (astaxanthin) is desirably stable in dark, at low pH, and at refrigerated temperatures. Hence it is concluded that it can be used in foods with lower pH packed in opaque packaging material and should be stored under refrigerated temperature for better stability and color retention in any foods.

References

[1] S. Ghosh, T. Sarkar, A. Das and R. Chakraborty **(2022)**. Natural colorants from plant pigments and their encapsulation: An emerging window for the food industry. LWT - Food Sci. Technol., **153:** 112527. doi: 10.1016/J.LWT.2021.112527.

[2] S. Ganguly, K. Bandyopadhyay, P. Dutta and A. Pramanik **(2019)**. A review on microbial-pigment: A good source of biocolour. Pharma Innov. J., **8:** 416-420.
[3] P. Bora, P. Das, R. Bhattacharyya and M. S. Barooah **(2019)**. Biocolour: The natural way of colouring food. J. Pharmacogn. Phytochem., **8:** 3663-3668.
[4] C. Gupta, A. P. Garg, D. Prakash, S. Goyal and S. Gupta **(2011)**. Microbes as potential source of biocolours. Pharmacology., **2:** 1309-1318.
[5] H. S. Tuli, P. Chaudhary, V. Beniwal and A. K. Sharma **(2015)**. Microbial pigments as natural color sources: current trends and future perspectives. J. Food Sci. Technol., **52:** 4669-4678.
[6] S. Samyuktha and S. N. Mahajan **(2016)**. Isolation and identification of pigment producing bacteria and characterization of extracted pigments. Int. J. Appl. Res., **2:** 657-664
[7] Z.-C. Hu, Y. G. Zheng, Z. Wang and Y. C. Shen **(2007)**. Production of astaxanthin by *Xanthophyllomyces dendrorhous* ZJUT46 with fed-batch fermentation in 2.0 M^3 fermentor. Food Technol. Biotechnol., **45:** 209-212.
[8] V. Monishdeep, H. W. Deshpande, A. Poshadri and B. V. P. Kumar **(2022)**. A review on potential microbial sourced bio-colours from fungi. Pharma Innov. J., **11(Special Issue):** 3200-3205.
[9] N. F. Haard **(1988)**. Astaxanthin formation by the yeast *Phaffia rhodozyma* on molasses, Biotechnology Lett., **10:** 609-614.
[10] J. D. Fontana, M. F. Guimaraes, N. T. Martins, C. A. Fontana and M. Baron **(1996)**. Culture of the astaxanthinogenic yeast *phaffia rhodozyma* in low-cost media. Appl. Biochem. Biotechnol., **57-58:** 413-422.
[11] J. D. Fontana, D. A. Mitchell, O. E. Molina, A. Gaitan, T. M. B. Bonfim, J. Adelmann and A. Grzybowski **(2008)**. Starch depolymerization with diluted phosphoric acid and application of the hydrolysate in Astaxanthin fermentation. Food Technol. Biotechnol., **46:** 305-310.
[12] M. Vázquez, F. Diniz and A. M. Martin **(1996)**. Single-cell protein from *Xanthophyllomyces dendrorhous* yeast: continuous fermentation using peat extract as substrate. Ciencia Tecnol. Alime., **2:** 12-16.
[13] M. Olaizola **(2000)**. Commercial production of astaxanthin from *Haematococcus pluvialis* using 25,000-liter outdoor photobioreactors. J. Appl. Phycol., **12:** 499-506.
[14] J. Ramırez, M. L. Nunez and R. Valdivia **(2000)**. Increased astaxanthin production by a *Phaffia rhodozyma* mutant grown on date juice from Yucca fillitera. J. Ind. Microbiol. Biotechnol., **24:** 187-190.
[15] C. H. Luna-Flores, J. J. Ramrrez-Cordova, C. Pelayo-Ortiz and E. J. HerreraLopez **(2010)**. Batch and fed-batch modeling of carotenoids production by *Xanthophyllomyces dendrorhous* using *Yucca fillifera* date juice as substrate. Biochem. Eng. J., **53:** 131-136.
[16] A. R. Dominguez-Bocanegra and J. A. Torres-Muñoz **(2005)**. Astaxanthin hyperproduction by *Phaffia rhodozyma* (now *Xanthophyllomyces dendrorhous*) with raw coconut milk as sole source of energy. Appl. Microbiol. Biotechnol., **66:** 249-252.
[17] R. J. Stoklosa, D. B. Johnston and N. P. Nghiem **(2019)**. Phaffia rhodozyma cultivation on structural and non-structural sugars from sweet sorghum for astaxanthin generation. Process Biochem., **83:** 9-17.
[18] A. Torres-Haro, A. Gschaedler, J. C. Mateos-Díaz, E. J. Herrera-López, R. M. Camacho-Ruíz and M. Arellano-Plaza **(2021)**. Improvement of a specific culture medium based on industrial glucose for carotenoid production by *Xanthophyllomyces dendrorhous*. Processes, **9:** 429. doi: 10.3390/pr9030429.
[19] K. Jirasripongpun, W. Pewiong, P. Kitraksa and C. Krudngern **(2008)**. Carotenoid production by *Xanthophyllomyces dendrorhous*: use of pineapple juice as a production medium, Lett. Appl. Microbiol., **47:** 112-116.
[20] N. Ananda and P. V. Vadlani **(2010)**. Production and optimization of carotenoid-enriched dried distiller's grains with solubles by *Phaffia rhodozyma* and *Sporobolomyces roseus* fermentation of whole stillage. J. Ind. Microbiol. Biotechnol., **37:** 1183-1192.

[21] B. Stachowiak **(2012)**. Astaxanthin synthesis by yeast *Xanthophyllomyces dendrorhous* and its mutants on media based on plant extracts. Indian J. Microbiol., **52:** 654-659.
[22] C. Yuan, L. Du, Z. Y. Jin, X. M. Xu Storage stability and antioxidant activity of complex of astaxanthin with hydroxypropyl-β-cyclodextrin, Carbohydr. Polym., **91:** 385-389.
[23] Q. Shen and S. Y. Quek **(2014)**. Microencapsulation of astaxanthin with blends of milk protein and fiber by spray drying. J. Food Eng., **123:** 165-171.
[24] P. C. Mezquita, B. E. B. Huerta, J. C. P. Ramírez and C. P. O. Hinojosa **(2015)**. Milks pigmentation with astaxanthin and determination of colour stability during short period cold storage. J. Food Sci. Technol., **52:** 1634-1641.
[25] F. Tamjidi, M. Shahedi, J. Varshosaz and A. Nasirpour **(2018)**. Stability of astaxanthin-loaded nanostructured lipid carriers in beverage systems. J. Sci. Food Agric., **98:** 511-518.
[26] M. Vakarelova, F. Zanoni, P. Lardo, G. Rossin, F. Mainente, R. Chignola and A. Menin et al., **(2016)**. Production of stable food-grade microencapsulated astaxanthin by vibrating nozzle technology. Food Chem., **221:** 289-295.
[27] A. Bassijeh, S. Ansari and S. M. H. Hosseini **(2020)**. Astaxanthin encapsulation in multilayer emulsions 1283 stabilized by complex coacervates of whey protein isolate and Persian gum and its use as a 1284 natural colorant in a model beverage. Food Res. Int., **137:** 109689. doi: 10.1016/j.foodres.2020.109689.
[28] N. Anarjan and C. P. Tan **(2013)**. Chemical stability of astaxanthin nanodispersions in orange juice and skimmed milk as model food systems. Food Chem., **139:** 527-531.
[29] N. Anarjan and C. P. Tan **(2013)**. Effects of storage temperature, atmosphere and light on chemical stability of astaxanthin nanodispersions, J. Am. Oil Chem. Soc., **90:** 1223-1227.
[30] S. R. Mhalaskar, S. S. Thorat and A. A. Kulthe **(2018)**. Stability analysis of food bio-colour extracts from broken rice through solid state fermentation. Int. J. Pure App. Biosci., **6:** 304-313.