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BIO-UTILIZATION OF INDUSTRIAL WASTE: CHARACTERIZATION AND ANTIOXIDANT ACTIVITY

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ABSTRACT

Peels, seeds, liquids, and molasses are among the waste products produced by the fruit and vegetable processing industries. These waste products are rich in carbohydrates, proteins, fibers, vitamins, and minerals. Fermentation can be used to produce colors from this waste. Our trash disposal concerns are solved and pollution in the environment is reduced by reusing these materials. Solid-state fermentation of Blakeslea transport (+) MTCC 884 was used. Orange, carrot, and papaya peel waste can be used to mine and optimize environmental elements for the formation of carotene. All factors had a significant impact on -carotene production, with a determined yield of 0.125-0.128 mg/mL. A variety of techniques were used to characterize the extracted color, including High-Pressure Liquid Chromatography (HPLC), LC-MS, mass spectroscopy, and FTIR. (Rt 13.37) was confirmed by mass spectroscopy of the extracted color, which yielded 537.608 as the mass/charge ratio (m/z). A further 76 percent of these fruits and vegetable wastes contained -carotene, suggesting that they could be used to produce pure carotene. DPPH and ABTS tests indicate that it has good antioxidant capabilities.

Keywords: Fruit Peel Waste, Fermentation, HPLC, FTIR, UV-VIS Spectroscopy.

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INTRODUCTION

Bio colors derived from microorganisms are called micro colors.[1] The use of food colors has become increasingly popular due to technological advancements and a growing awareness of food safety among consumers.[2] Food colors are a safer alternative to artificial non-food-grade coloring agents. As a result of their natural occurrence, carotenoids, in the form of hydrocarbons, give fruits and vegetables their distinctive coloration. They are lipid-soluble pigments that range in color from yellow to red, affecting the acceptance of numerous foods.[3] Commercially available carotenoids include -carotene, lycopene, and capsanthin, among others. Products like carotenoids are in high demand, and their prices are rising as well. Chemical synthesis produces the majority of commercially available carotenoids; however, they can also be obtained from agricultural sources. Some fungi, yeast, and algae are utilized in bio-technological and microbiological methods to produce carotenoids. Fungi are the best microbial sources of carotenoid production for solid-state fermentation, as they require less moisture.[4] Blakeslea transport, a filamentous fungus, produced the most carotene.

Agro-industrial wastes can be used as cheap carbohydrate sources for fermentation-based carotenoid production, which helps reduce environmental degradation by utilizing waste.[5] Utilizing low-cost agricultural byproducts as a rich source of sugar, fat, minerals, organic compounds, and mineral compounds, biotechnological techniques are used to increase carotenoid production by reducing processing costs.[6,7]

The goal of this research is to assess the possibility of fruit and vegetable peel discarded as a source for solid-state fermentation employing Blakeslea transport to produce carotenoids, as well as to boost the fermentation media confirmation for higher carotene yields. The extracted color is examined and described using UV-Spectrophotometry, Colorimeter, HPLC, FTIR, LCMS, and MS.DPPH and ABTS methods are used to check the color's antioxidant activity.[8-10]

EXPERIMENTAL

General Chemicals

Ranked Chemicals, Mumbai, India, provided the YpSS media components (contents are food-grade starch, magnesium sulfate, yeast extract, and di-potassium hydrogen orthophosphate) Blakeslea trispora

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organisms are maintained and propagated using this medium.[11] In addition to asparagine and malt extract, Merck Chemicals provided the ingredients. In India, Sigma–Aldrich Limited provided the standard -carotene for the study.[12] It was obtained from Fluka (Buchs, Switzerland). Methanol, ethyl acetate, and acetonitrile, all HPLC and LCMS grade solvents, were used.

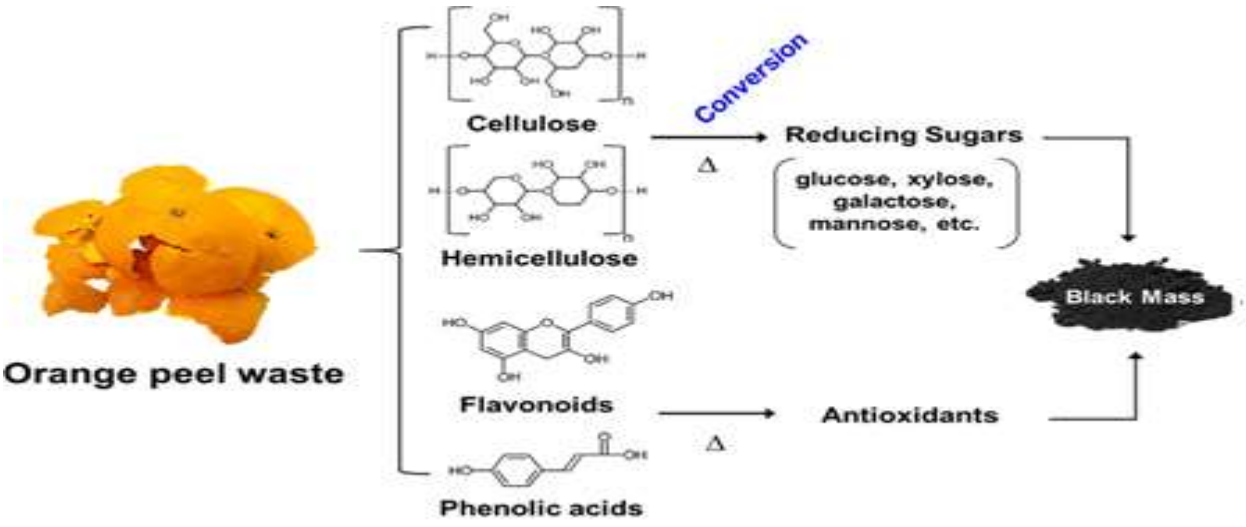

Fig.-1

Microorganisms and Culture Conditions (Microbiology and Culture)

Blakeslee transport MTCC 884(+) mating type freeze-dried culture was obtained from IMTECH Chandigarh, India.[13] Cultures were lyophilized and reactivated using (YpSS). Incubation took place at 25°C for 7 days, with slant tubes kept at 4°C.

Sources of Fermentative Substances

Local fruit markets in Jaipur were combed for fruit peels and vegetables. To make use of the peels, they were dried and ground down to a very fine particles size less than 0.5 mm. During fermentation, this fine substance is utilized for the growth of bacteria.[14,15] As a carbon and nitrogen source, these peels are added to the fermentation medium. The carbohydrate, nitrogen, and protein content of the powder were determined using a proximate analysis.[16]

Fermentation

Blakeslee transport (+) strain was cultured on (GAY) fermentation medium. Substrat (fruit and vegetable waste) was diluted at 1:5 with a moistening medium in conical flasks.[17] Autoclaving and incubation for 24 hours were followed by inoculation of 106 spores of 7-day-old Agar Slant in one loop on the inoculation media. The medium was fermented for four days at 28°C at 200 rpm.[18-20]

Fruit and Vegetable Waste Fermentation Medium Optimization

Blakeslee transport was used in a series of experiments to performed fermentation conditions favored substrates were agro-industrial wastes that could lower production costs and reduce contamination rates of fungal developed on an industrial scale.[21,22] The effects of H/OH ions concentration, Fermentation temperature, incubation time, and nitrogen component addition on -carotene yield were studied. According to the previous results, each subsequent experiment was designed.

Optimization of pH

Substrates of 0.5mm particle size were used in different Erlenmeyer flasks to optimize fermentation medium pH. (250 mL).[23] This study examined changes in carotene production in fermentation flasks with pH ranges of 5, 6, 6, 7, 7, 8, and 9.[24-26] For maximum carotene output, a pH range of 6.20-7.20 was investigated. The results were encouraging.

Variable Incubation Time and Temperature
New Brunswick Scientific, NJ, USA provided an incubator (Innova 4230) for 10 days to analyze the natural shape of Blakeslea trispora for biomass and -carotene biosynthesis

Experimental Findings
β-carotene Determination
Using a solvent extraction method, a pigment was collected from the fermentation media after four days of fermentation.[27] To estimate -carotene, spectrophotometers are used. A 1:1 ratio of material used in fermentation to petroleum ether was used in the -carotene assay. Consequentially for 2 hours at 200 pm and 28°C in a shaker incubator. This mixture of petroleum ether extracts was then measured for -carotene absorbance at 449 nm. It is possible to vary the concentration to obtain a -carotene standard graph.

Detection of β-carotene by HPLC
It was measured by using HPLC (High-Performance Liquid Chromatography) that -carotene in the extracts was pure. For the standard curve, a 10mg/100mL stock solution of standard beta-carotene was prepared, and then successive dilutions of the stock solution were performed. An isocratic HPLC analysis (Shimadzu, Japan) was performed on the Merck C-18 column (250mm4.6mm, particle size 5m) at 25°C with a wavelength of 449nm. 76:12:12, v/v) Methanol, Acetonitrile, and Ethyl Acetate were the mobile phases, as fast as 2 ml min-1.

Study by FTIR
The infrared spectrum of the extracted color was measured at 25°C with a Tenso-28 FTIR (Bruker, USA) in the 500–4000 cm1 wavenumber range. Approximately 2 mg/0.5 mL of lyophilized powders and the liquid extract were placed on the ATR for determination. For each sample, the entire acquisition process took one minute.[28]

Study by LC/MS
The purified compound's mass spectrum was identified using LC/MS (Waters Corporation, U.K., Alliance 2795, Q-TOF Micromass). A Shimadzu HPLC system was used with a (Reverse Phase) C-18 column. Positive ion-spray MS was used. There were three mobile phases used (76:12:12) in liquid chromatography, and the mass was measured as fast as one scan/second electrospray (9.10 kV) with a positive electrospray technique.

Color Detection by Hunter Lab Colorimeter
Colorflex spectrophotometer was used to measure the color parameters L*, a*, b*. ColorFlex EZ model (Hunter Laboratory Made USA) used the advantage of 45º/0º guaranteeing that you don't just get your colors right, but that they're great every time you use them. It measures your sample in the same way as the human eye does, therefore it's quite accurate. Not only in a lab but in real life, it allows you to view your colors exactly as your customers do, not just in the lab. Black and white tiles were used to calibrate the instrument. I made sure that the sample was completely covered by the bottom of the sample dish by carefully filling it into the cup.[29] All samples were measured in triplicate, and the average value was computed.

A DPPH and ABTS Approach for Measuring Antioxidant Activity
The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to define the antioxidative activity of lyophilized color samples. Petroleum ether was used to prepare a standard -carotene stock solution (1g/mL). Individually, 100L of -carotene solutions (standard and extracted) were treated with DPPH (0.3 mM) in the same volume. The absorbance was measured at 517um of all samples after 30 minutes. The samples were kept in dark during this period By the same method, Thakur et al.[30] measured radical scavenging activity (percent) of ABTS. In methanol, a standard -carotene stock solution (1g/mL) was prepared. Test solutions (5–30 g/ml) were prepared by diluting a carotene sample in methanol before testing. 100L of the extracted sample solution was diluted with 3.6 ml of the ABTS solution (standard -carotene and the

extracted -carotene). For each sample, the absorbance was measured at 734 nm using a UV spectrophotometer. This activity could be estimated by applying the below equation:

Radical Scavenging Activity (%) = $\left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ control}\right)$ x 100

For the DPPH method, it was utilized to analyze the trend of -carotene's antioxidant impact over time.

RESULTS AND DISCUSSION

Physicochemical Study of Fruit Peels

The three different types of fruits namely banana, orange, lemon collected from fruit juice sellers as waste. Dry these fruit peel waste in sunlight for 15 days then crush in a mixer and convert into dry powder form. Then physicochemical analysis was carried out. Fruit peels were analyzed for trace metal content.

Table-1

Elements (mg/mL)	Orange	Lemon	Banana
Cd	0.24	0.93	0.19
Cr	1.03	0.14	1.39
Cu	1.27	1.42	2.11
Fe	112.9	7.23	33.9
Pb	0.23	0.03	0.65
Ni	1.22	0.72	0.41
Mn	4.75	0.55	3.59
Mg	14.25	19.28	2.55
Zn	10.45	14.49	4.72

The proximate and ultimate analysis is carried out for various fruit peel powders. Results are shown below in Table-2.

Table-2

Proximate Analysis of Fruits Peel					Ultimate Analysis of Fruits Peel				
FP	Moisture (%)	Ash (%)	Volatile Matter (%)	Fixed Carbon (%)	N (%)	C (%)	H (%)	S (%)	O % (by Difference)
BP	9.68	5.11	84.38	0.83	1.33	40.22	6.14	0.098	52.22
OP	7.84	5.19	85.9	1.07	1.16	38.87	6.19	0.11	53.64
CP	7.52	4.38	85.68	2.42	0.68	38.56	6.2	0.1	54.55
LP	6.23	5.46	86.18	2.13	1.24	40.28	5.96	0.19	52.25
JFP	6.54	6.23	86.22	1.01	0.96	40.11	5.86	0.12	53.08

Solid Substrates Characterization

Peel wastes were subjected to an elemental analysis, which revealed that carrot peel wastes had the highest carbon content. According to Fig.-1 the carbon found in carrots was 23.86 percent, and the nitrogen was only 0.886 percent. The ratio of C and N in carrot peel was therefore 27:1. Figure 1b shows that orange peel waste contains 22.56 percent carbon and 0.735 percent nitrogen, for a carbon-nitrogen ratio of 30:1. Following Gao et al. [31], these wastes provide the fungus Blakeslea trispora with an adequate carbon-nitrogen ratio for its growth.

According to Oikeh et al.[32], orange peel waste contains 41.25 percent carbohydrates and 3.5 percent protein. When it comes to carbohydrates, papaya peels contain 61.8 percent and carrot peels contain 1.5 percent, respectively. Papaya had also been the subject of similar research in the past. The Carbohydrate content in Peel waste was found in a larger amount

Various Factors for β-carotene Maximum Yield

Agro-industrial by-products at low cost are being used to produce microbial -carotene, and the composition of the fermentation medium and its environmental conditions are being optimized. Blakeslee trispora can grow and produce carotenoids on fruit peel waste, as shown in the results described below.

The use of agricultural waste materials instead of commercial protein and sugar sources has clear cost advantages, and waste disposal issues can be avoided. pH optimization for maximum β-carotene yield.[33] Microbes are highly active concerning the pH value of any solution.

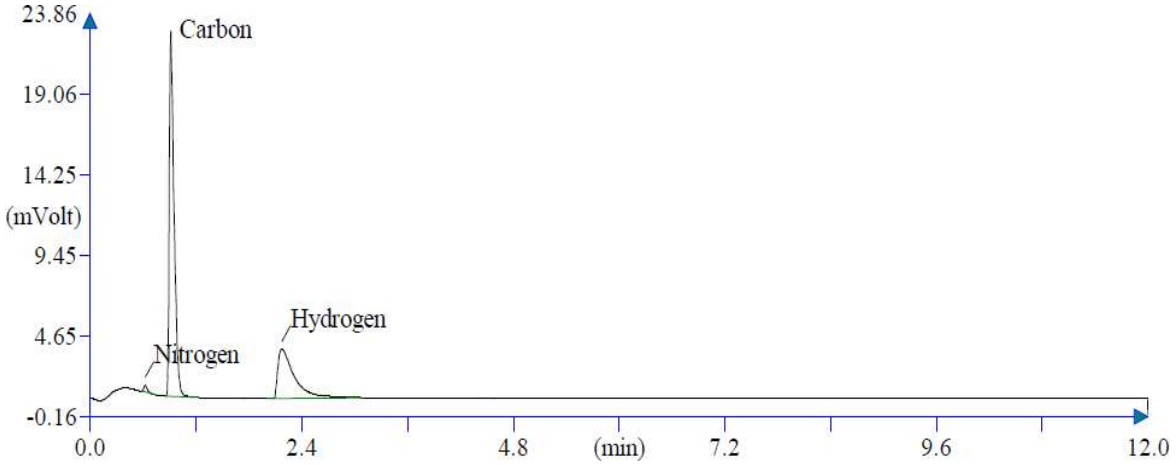

Fig.-2

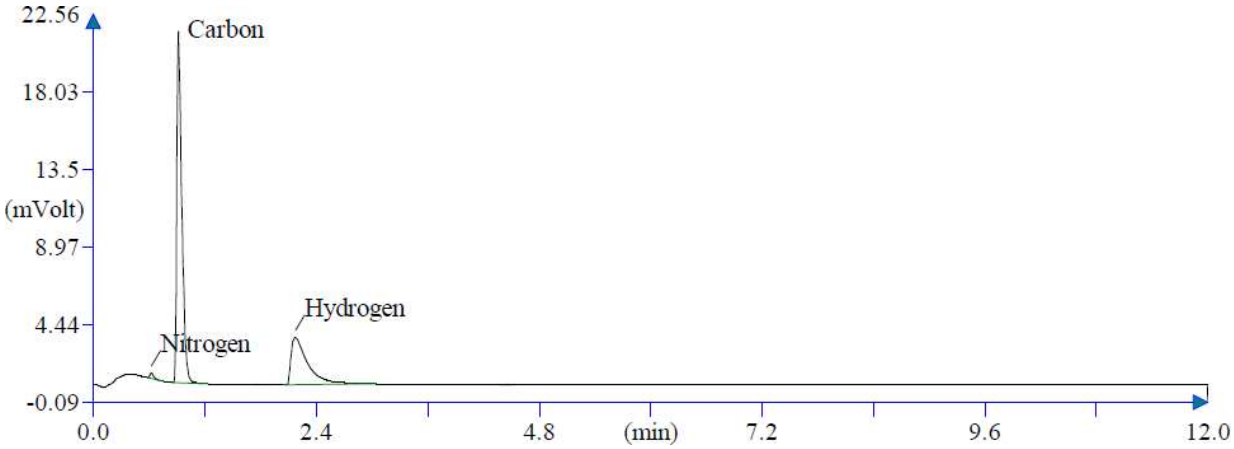

Fig.-3: Hydrogen Carbon and Nitrogen part of Peels Powder (2) Carrot Extract (3) Orange Extract

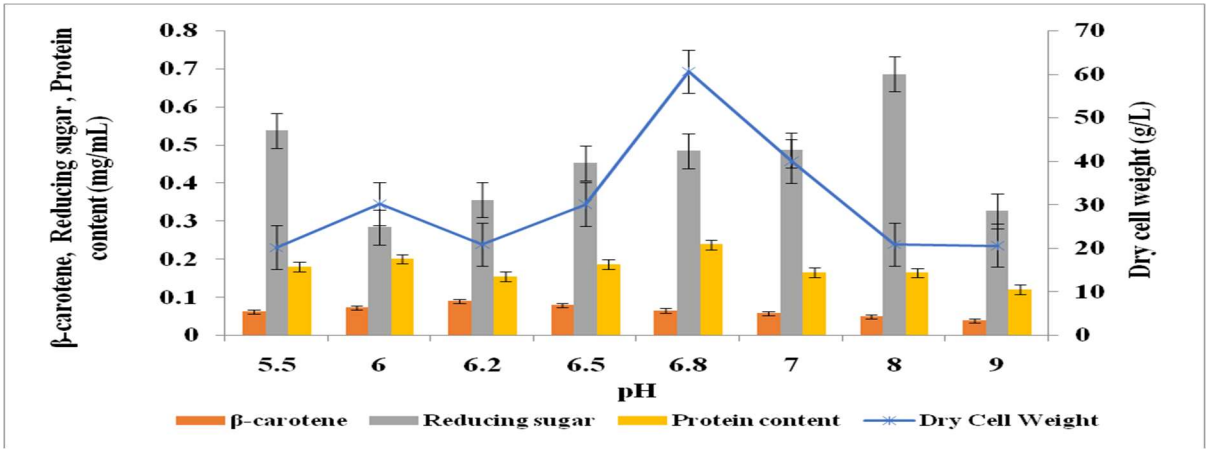

Fig.-4

It shows the effect of different pH levels on the production of beta-carotene, sugar reduction, protein, and dry cell measured (50 g/50 mL) (Fig.-2a). Fungus growth and carotenoids production were greatly

affected by pH. There were significant reductions in -carotene production at pH 5-5. A-carotene and biomass creation increased with pH increase and peaked at pH 6.2. Figure-2a shows that a greater yield was gained in the pH range of 6.2-6.5 as compared to pH 5-5 or pH 8-9, respectively.[34] According to Choudhari and Singhal, the Bl can produce 97.1 mg/L of -carotene at pH ranges of 6.2 to 6.5.

Due to an increase in biomass and carotene production, the number of residual sugars decreased during fermentation from 6.0 to 7.0. Blakeslee trispora fermented media at pH 6-7, according to Yekta Goksungur et al. At an initial pH range of 6.0-6.5, the highest concentrations of -carotene and biomass dry weight were observed. Blakeslee trispora produces more -carotene under alkaline conditions, as reported by Kim et al.[35]

Table -3

S. No.	Fruit Peel	pH measured
1	Alkaline	7.9
2	Pomegranate	4.8
3	Lemon	6.4
4	Citrate	6.1
5	Banana	7.9
6	Orange	5.8

Temperature Enhancement for β-carotene Synthesis

Temperature is another important factor that impacts cell proliferation and bio-pigment formation. In addition to temperature, cell growth and pigment formation are influenced by temperature. Figure-2b shows the temperature effect on carotene making, indicating that the microorganism is mesophilic. Up to 28°C, color production grew but declined as the temperature rise. Fermentation medium incubation temperature was revealed to be a significant factor in reducing sugars content and dry cell weight, leading to decreased synthesis of -carotene On the other hand, according to López-Nieto and colleagues, (28–30°C) was the optimal temperature for and carotenoids to be produced.

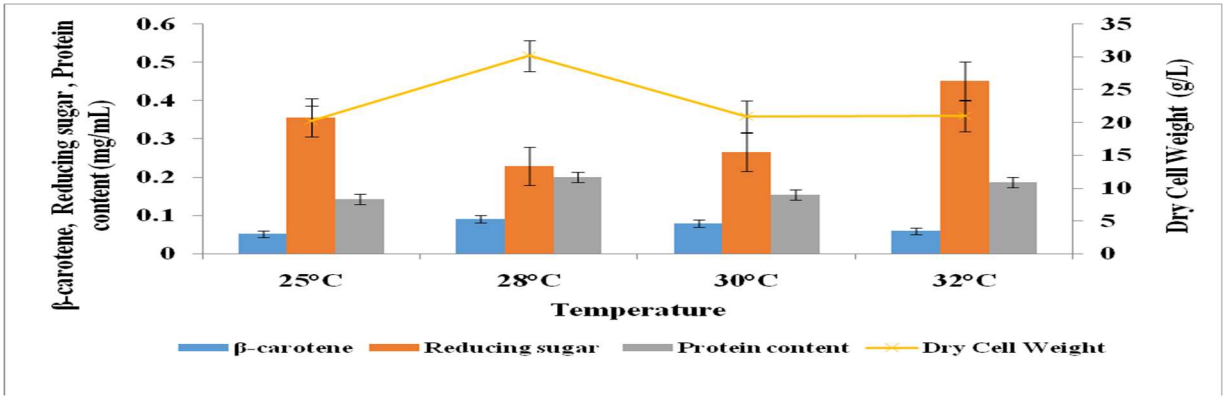

Fig.-5

Optimization of Nitrogen Sources

At a pH of 6.2 and an incubation temperature of 28°C, Blakeslea trispora produces pigments in response to different nitrogen sources. It was found that adding yeast extract to media containing fruit peel waste increased the pigment yield to its highest level. A promising pigment yield was observed in yeast extract (0.096 mg/mL), trailed by extract of protease. Case in produced the least amount of carotene color.

Incubation Period Optimization for β-carotene Synthesis

It shows the fermentation kinetics of cells growing in the fruit waste and producing -carotene. Carbon and energy sources were consumed as cell mass grew by the organism as sugars in the medium were consumed.[36] After 24 hours of fermentation, cell mass production began and peaked at 72 hours. After 12 hours, -carotene production began, and the maximum yield was reached after 96 hours of fermentation. As a result, after 96 hours a maximum -carotene production of 0.127 mg/mL. As a result, our results are very similar to Choudhari and Singhal's growth curve for -carotene (1382 mg/L) in synthetic media.

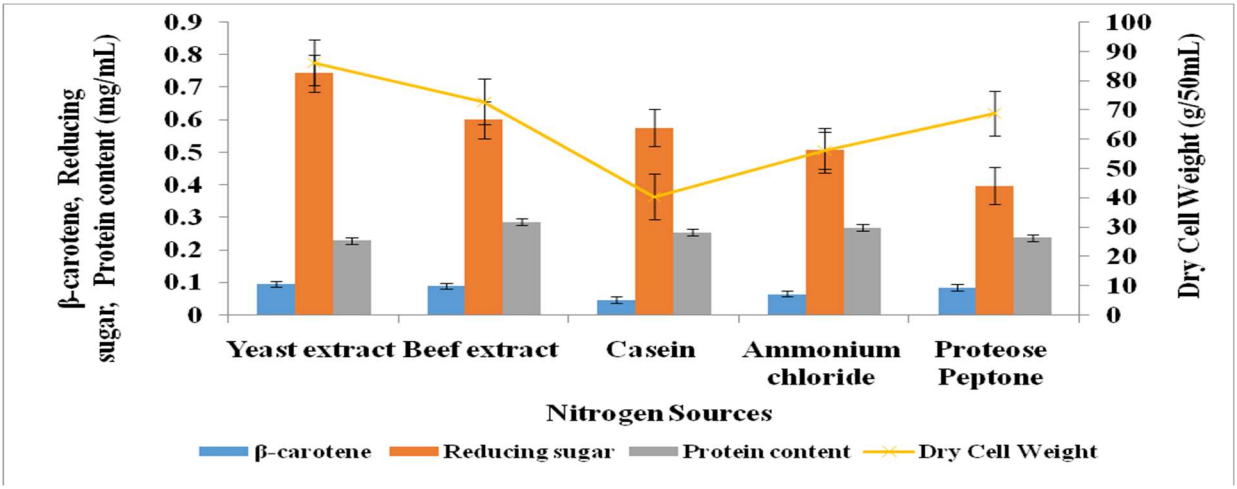

Fig.- 6

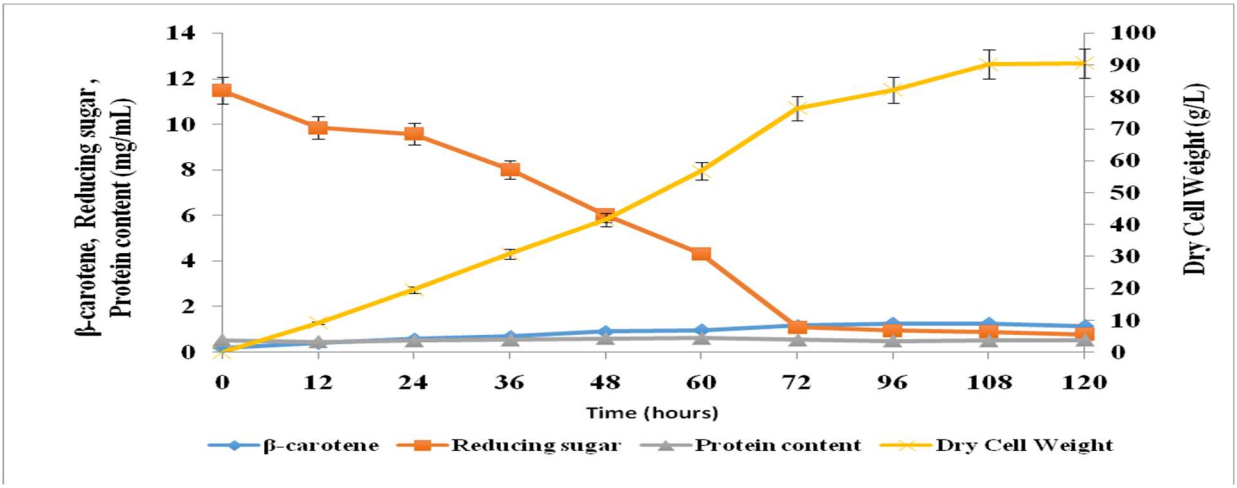

Fig.-7

Color Extraction

Colorimeter Analysis of Extracted Colors

The carotenoid group was identified by colorimeter analysis of the extracted color in the 200-800 nm wavelength range. The presence of carotene was confirmed by the sharp peaks at 449 nm. As shown in Fig.-3a, the absorbance peaks of both stock carotene and extracted sample colors were found to be present.

HPLC (High-Performance Liquid Chromatography)

Figure-3b Carotene standard HPLC chromatogram in gradient elution system, as shown in Fig.-3b. Using a standard -carotene retention time as a reference, Fig.-3c identifies and quantifies the peak. It was determined that the -carotene sample was extracted after 13.198 and 14.46 minutes. The relative retention times of stock carotene were used to confirm the peaks' identities.

Thus Also in agreement with Bhosale and Gadre, -carotene was extracted and HPLC analysis of the extracted sample was.

Fourier Transform Infrared Spectroscopy Analysis of An Extracted Sample

Figure-4 shows the FTIR spectrum of -carotene purified to a standard (500-4000 cm1)[37]. After isomerization, -carotene's configuration changes from its natural state. Alkyl C-H group stretching modes

were determined by absorption bands between 2900 and 3050 cm-1 and aromatic C-H group stretching modes were determined by bands at 2874 and 2827cm-1. While the C-H group's asymmetric deformation mode was revealed by the high-pitched peaks at 1445-1455 cm1, the symmetric distortion mode was revealed by a band at 1362 cm1. The trans conjugated alkene -CH=CH- out-of-plane deformation mode was visible at lower frequencies, such as 967 cm1.

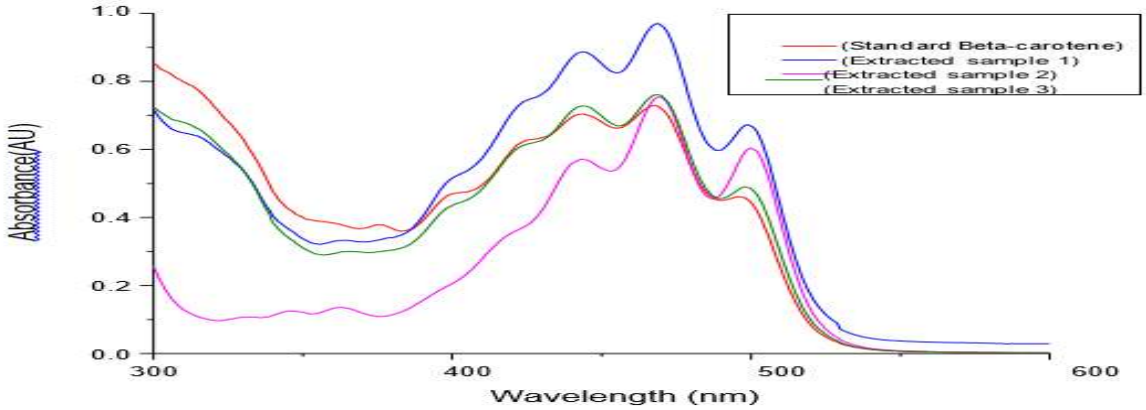

Fig.-8

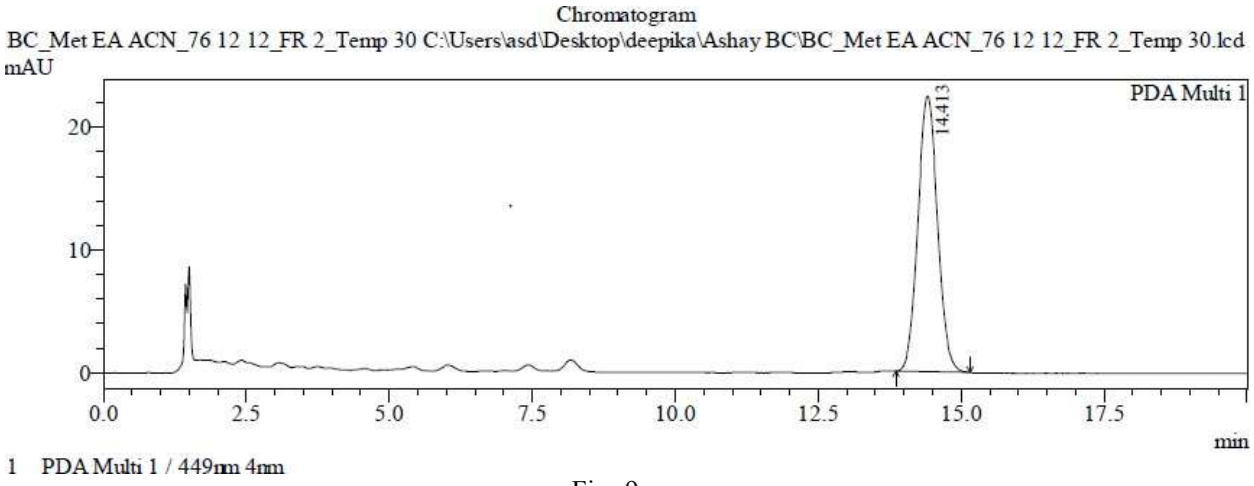

Fig.-9

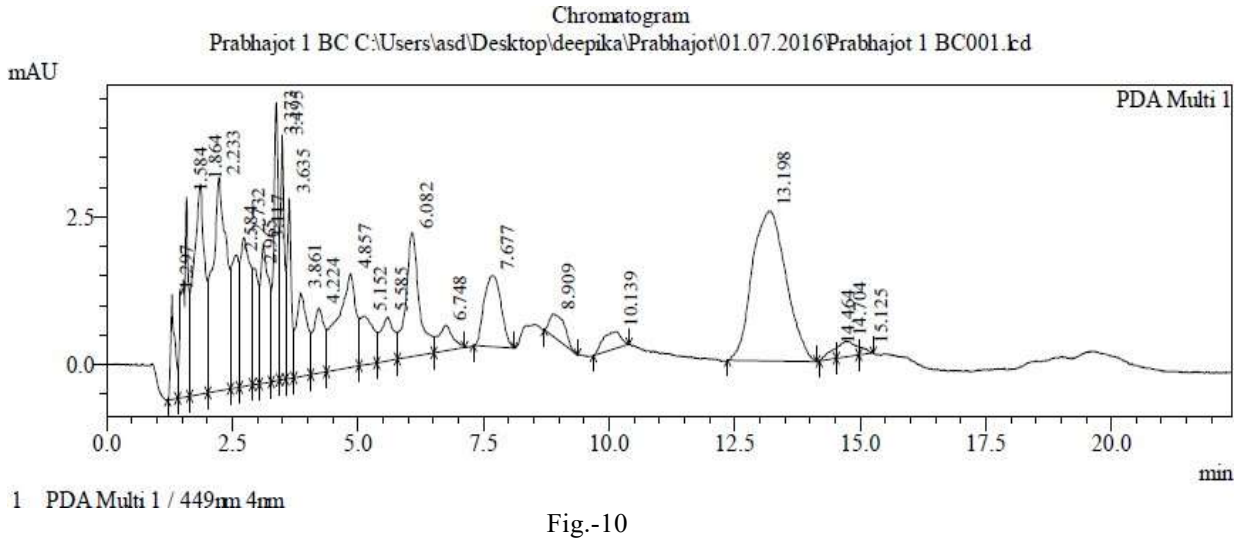

Fig.-10

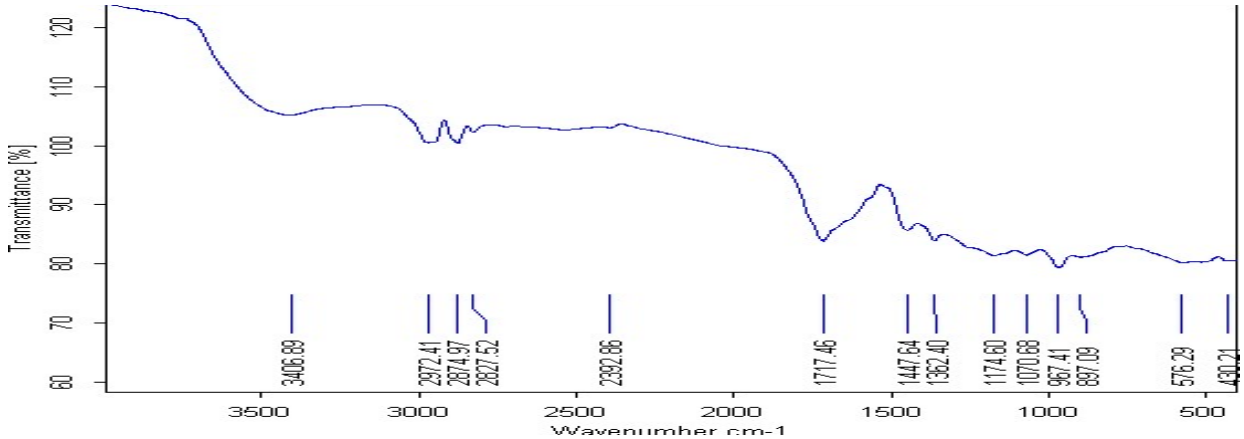

Fig.-11

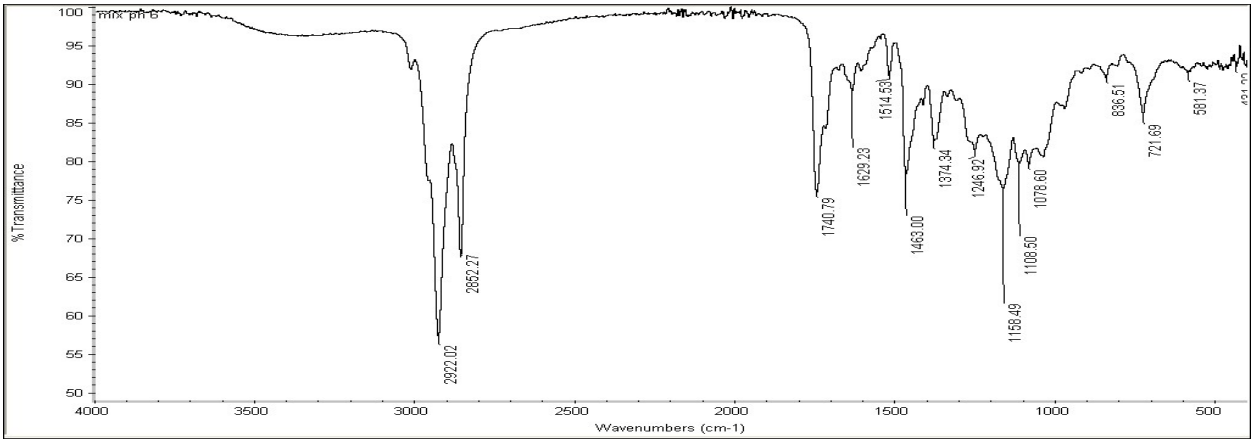

Fig.-12

The FTIR analysis of the extracted color sample is shown in Fig.-4. The -carotene trans -CH=CH- absorption band at 3005cm1 was weak. 2922 cm-1 and 2852.27 cm-1 were the absorption peaks for the alkane group, and 1463 cm-1 was the absorption peak for the aromatic ring. As well as C=O stretching vibrations, the 836 cm-1 peak is indicative of (=CH) bonds in alkene groups. C=O stretching peaks at 1740 cm-1 were observed in the extracted color spectrum. The mass spectrum of the color was determined by performing an MS analysis on the extracted pigment.

Identification of Color

As shown in Fig.-5, different fermentation time intervals produce different L*, a*, and b* values of color. L* (lightness) of the color increased with time and then decreased slightly after 96 hours of storage. Accordingly, fermentation at 96 hours resulted in the highest and most significant color production. From 12 to 60 hours, the value of a* was (P0.05) much higher than that of b*. During fermentation, 60-96 hours were needed to achieve the desired color.

DPPH and ABTS Method Validation for Antioxidant Activity

Figure-7 shows Antioxidant functional activity can be evaluated using the colorimetric DPPH method.[38] About 86 percent of the antioxidant activity of standard carotene was still present after 90 days. As an alternative, a carotene sample that was extracted and optimized had an antioxidant activity of 83 percent, but its effectiveness declined after 90 days. At 0 time, the scavenging activity of standard and extracted samples differed by only 3 percent. As a standard, Trolox was used to determining -carotene's antioxidant activity using the ABTS method. A comparison of the extract's radical scavenging activity to that of the standard was made at 0 days and 7 days. As a result, carotene is a powerful antioxidant because of its ability to neutralize free radicals and oxygen quenching capabilities.

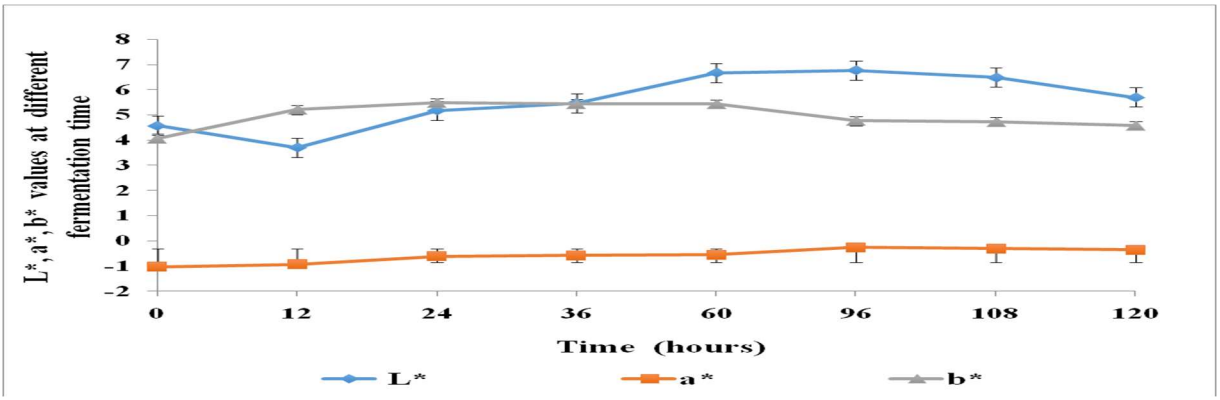

Fig.-13

CONCLUSION

This study indicated that Fruit and vegetable waste as substrate allowed Blakeslea trispora to grow with a high yield of -carotene, compared to artificial media with other agricultural waste such as cabbage, watermelon husks, and peach skins. It is therefore possible to produce total carotenoids from fruit and vegetable waste, reducing the need for other expensive medium components and alleviating waste disposal issues. In this study, we found that agro-food waste might be used to produce high-purity carotene on a wide scale for a low cost. LCMS, FTIR, and HPLC were used to characterize the colors, demonstrating their purity. -carotene can also be used therapeutically due to its strong antioxidant properties, which last for over 90 days.

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