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Extraction of Beta Carotene from Pineapple (*Ananas comosus*) Peels Using *Escherichia coli*

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ABSTRACT

A simple method for production of beta carotene using cheap raw material of fruit peels of (*Ananas comosus*) pineapple was developed in our laboratory. Different parameters like temperature, production time was studied extensively and Beta carotene was extracted by counter current solvent extraction method. Yield was found to be 0.562µg/ml. This method is very much suitable for small scale industry with waste collected from local markets.

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INTRODUCTION

Pineapple *(Ananas comosus)* is an important fruit crop in India. Originated in Brazil, it has spread to other tropical parts of the world. A good source of vitamins A and B, pineapple is fairly rich in vitamins C, calcium, magnesium, potassium and iron. It is also a source of bromelain, a digestive enzyme. Kew, Giant Kew, Charlotte, Roth child, Queen, Mauritius, Jaldhup and Lakhat are various varieties in pineapple[1].

Hydrocarbon carotenoids are classified as carotenes, derived from the Latin name "carrots". Approximately 50 carotenoids known as "provitamin A compounds" considered as the precursor for retinol, an active form of vitamin A. Among the commonly occurring carotenoids such as α-carotene (alpha carotene), β-carotene (β-carotene) and lycopene, β-carotene is one of the most commonly occurring carotenoids[2].

Beta-Carotene is being used often in food industry as food additive and it is the most suitable formulation for many foods preparations. Addition of beta-carotene gives excellent stability in food products during processing and storage[3].

Being an important antioxidant β-carotene protects cells from damaging effects of free radicals, due to its high radical scavenging activity[4]. It enhances the functioning of immune system, reduces the chances of cancer and heart diseases and a precursor of vitamin A.

The bright colours found in nature and the molecules which cause them, have always fascinated organic chemists. The earliest studies on carotenoids date back to the beginning of the 19th century. Beta-carotene was first isolated by Wackenroder in 1831, and many other carotenoids were discovered and named during the 1800s, although their structures were still unknown.

Beta carotene extraction was done from various substrates like whey ultra filtrate[5], n hexane , n dodecane[6], vegetable feedstock oil palm[7], crude olive pomace oil and crude soya bean oil[8], molasses[9], industrial glycerol[10] and glycerol[11] using various organisms like *E. coli* YJM49, *E. coli, E. coli* MG1655, *Blakeslea trispora* NNRL 2895/2896, *Blakeslea trispora* Wild type F921/F986, *Blakeslea trispora, filamentous fungi, microalgae, yeast, and Rhodosporidium toruloides* NCYC 921.

MATERIALS AND METHODS

BETA CAROTENE PRODUCTION

Preparation of standard curve: Twenty five milligram β-carotene was dissolved in 2.5mL chloroform and volume was made up to 250mL with chloroform and 10mL of this solution was diluted to 100mL with 5, 10, 15, 20, 25 and 30mL of this solution was taken in volumetric flask each containing 3ml of acetone. The concentration was 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 micrograms (µg) of β-carotene/mL after serial dilution. Absorbance recorded as Optical Density (O.D) at 452nm was plotted against the β-carotene concentration

Fermentation medium:

Carbon source – pineapple peel- 100gm taken

K_2HPO_4	9.8g/L
Beef extract	5g/L
Ferric ammonium Ciliate	0.3g/L
Citric acid monohydrate	2.1g/L
$MgSo_4$	0.06g/L
Trace element solution	1ml
$NH_4\ MO_7O_{24}\ 4H_20$	0.37G/L
$ZNSO_4.7H_20$	0.29F/L
H_3B0_4	2.47g/L
$C_4SO_4.\ 5H_2O$	0.25g/L
$MNCL_2,\ 4H_20$	1.58g/L

100ml of broth containing K_2HPO_4, KH_2PO_4, $MgSO_4$, $MnSO_4$ was prepared, sterilized and 2 loops full of inoculum was inoculated. For the isolation of *E.coli* panipuri water was used as a source.

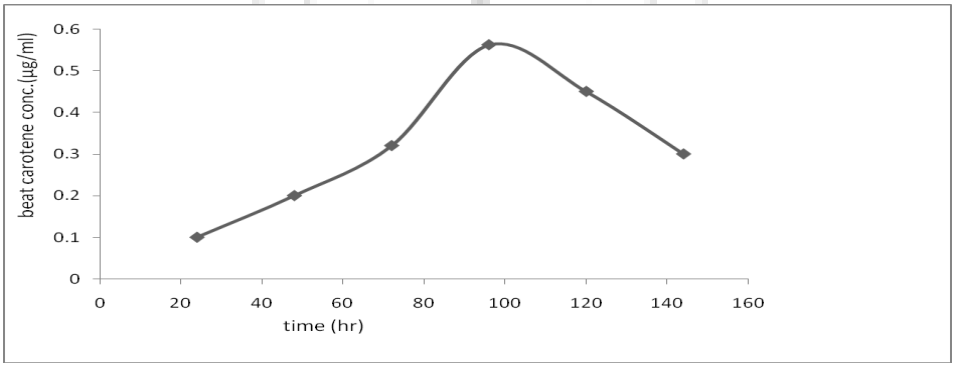
Raw materials i.e. pineapple waste was collected, weighed, washed, crushed, and then total volume made up to 100ml with water and transferred to 500ml conical flasks. Macro and micronutrients containing $MgSO_4$ (0.06g/L), KH_2PO_4 (9.8g/l), beef extract (5g/l), ferric ammonium citrate (0.3g/l), citric acid monohydrate (2.1g/l) and micronutrients

$(NH_4)_6MO_7O_{24}.4H_2O$. (0.37g/l), $ZNSO_4.7H_2O$ (0.29g/l), H_3BO_4 (2.47g/l), $CUSO_4.5H_2O$ (0.25g/l), $MNCL_2.4H_2O$ (1.58g/l) were dissolved in 100ml of water and 1ml solution of this was added to ground pulp and autoclaved at 121^0C and 15lb pressure for 20 minutes. The *E.coli* strain is inoculated in the media. Shake flasks are now placed on orbital shaker. The production was found to be maximum after 4 days.

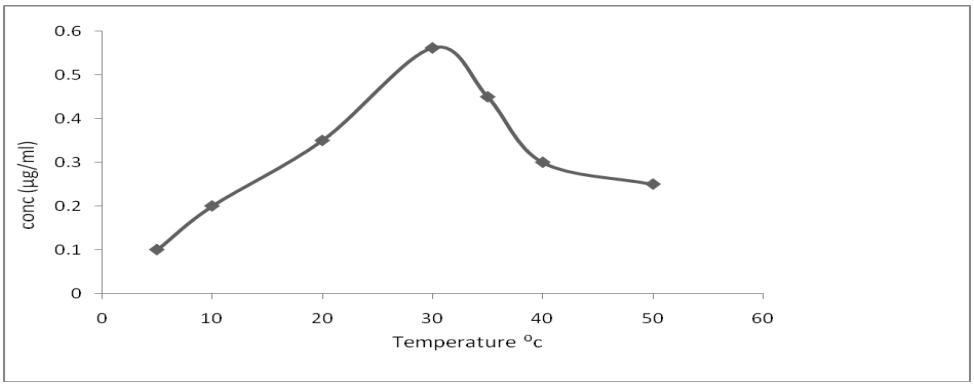
Measurement of β-Carotene

Cells were harvested by centrifugation at 6000rpm for 20 min and washed with sterile water. The cell pellet was then resuspended in acetone (1mL) and incubated at 55^oC for 15 minutes in dark. Samples were then centrifuged at 6000rpm for 20min and Acetone Supernatant containing β-carotene was transferred to new tube. Deduced the concentration of beta carotene from the standard curve. The carbohydrate present in raw material was estimated using Anthrone method. The concentration of sugars from standard graph was found to be 12%.

EFFECT OF TIME ON CONCENTRATION OF BETA CAROTENE



EFFECT OF TEMPERATURE ON CONCENTRATION OF BETA CAROTENE



RESULTS AND DISCUSSION

Literature survey reveals, that organic substrates like whey ultrafiltrate, n dodecane, n hexane, molasses, olive pomace oil, vegetable feedstock oil, crude soya bean oil were used for extraction of β-carotenes.

Among all the sources Corn was found to contain very high conc. of β-carotene. But in present method, fruit waste material of *Ananas comosus* which grows abundantly in and around Visakhapatnam was selected for production.

CONCLUSION

The optimum conditions for the productions were found to be 30°C temperature, pH 7.0 and 96hrs. This is a cheap raw material and the method was found to be cost effective. 100gm of waste produces nearly 0.562µg/ml of β carotene. This can be extracted by simple downstream processing method and can be added to food & fodder.

REFERENCES

1. http:hillagric.ac.in/edu/co/horticulture/lecture/hort-351-lecture-32.
2. Sergio A.R. Paiva MD, PhD & Robert M. Russell MD ;beta carotene and other carotenoids as anti oxidants; journal of American college of nutrition ;
Vol 18, issue 5, pages 426-433; DOI:10.1080/07315724.1999.10718880
3. H. Kläui and J. C. Bauernfeind, carotenoids as food colours ;Copyright © 1981 by Academic Press, ISBN 0-12-082850-2
4. Fujisawa ,S, M.Ishihara and Y. Kadoma 2400 ,Kinetics of radical scavenging activity of beta carotene related compounds.SAR QSAR Environ, Res., 15:33-41.
5. Ginka I. Frengova, Simova D. Emilina, and Dora M. Beshkova Carotenoid Production by Lactose-Negative Yeasts Co-Cultivated with Lactic Acid Bacteria in Whey Ultra filtrate received December 27, 2002/February 5, 2003
6. FangXu, Qi- peng yuan improved production of lycopene and beta carotene by *Blakeslea trispora* with oxygen vectors ,Process biochemistry vol 42, issue2, Feb 07 pg 282-293.
7. Maria Alice , Bernado Dias, Daniel Weingart ;Technological aspects of beta carotene production; Food and bioprocess technology July'11 vol-4 issue 5 pg.693-701.
8. Fani M ,Maria Z; carotenoid pattern in *Blakeslea trispora* grown on oil enriched media substrate with regard to triglycerol species accumulation ;European journal of lipid science & technology.
9. Gokseengus.Y, Mantzouridou. F; optimization of production of beta carotene from molasses by Blakeslea trispora;27 June 2002 ; DOI 10:1002/JCTb 662
10. Fani, Eleni ; Industrial glycerol as a supplementary carbon source for beta carotene production ;Jagri food chems 2008 pg 2668-2675.

11. Jianming Yang and Lizhong Guo, Biosynthesis of β-carotene in engineered E. coli using the MEP and MVA pathways Yang and Guo Microbial Cell Factories 2014, 13:160 http://www.microbialcellfactories.com/content/13/1/160

BIBLIOGRAPHY

- Sadaf.J, Tariq.M, Shehla.S, Saima.T, Asama.S,Shahid.J, Kashif.S, and Sartaj.A; comparative study for the extraction of beta carotene in different vegetables;
- Pakistan journal of nutrition 12(11) I 983-989, 2013 ISSN 1680-5194;Asian Network for scientific information, 2013.
- Improved production of carotenes from synthetic medium by Blakeslea trispora in bubble column reactor Biochemical engineering journal vol 67; 15 Aug'12 pg:203-207.
- SteenBock.H, Gross E G (1919); Fat soluble vitamin. II. The fat soluble vitamin content of roots together with some observation on their water soluble vitamin content, J Biol chem. 1919;40:501-531
- Paul d, Gerhard S ;carotenoid synthesis and phytoene synthase activity during mating of Blakeslea trispora ;Phyto chemistry vol.76, April'12 pg; No-45.
- Jin feng, xiu- ji lin, rui- sang liu ; optimization of mated fermentation production of lycopene by Blakeslea trispora NRRL 289(+), NRRL 289(-);Bioprocess and bios stem engineering May'12 vol 35 issue 4; pg 553-564.
- Sand man .G, N. Misawa ; fungal carotenoids ;Industrial Application Nycota Vol 10'02 pg 247-262.
- Seon-Wong nkim, Weon-taek seo, Young-hoon park; enhanced production of beta carotene from Blakeslea trispora span 20; biotechnology letter 1997, vol19 issue 6,pg:557-560
- Bina J,Irina , Enrique cerda; mutants and inter sexual hetero karyons of Blakeslea trispora for beta carotene production;july 2003 vol 69 ; pg 4043-4048
- Vera kuziana, Enrique; Modification of sexual development and carotene production by acetate and other carboxylic acid in Blakeslea trispora and P. blakelanus;Applied environment Microbiology July'06 vol 72 pg: 4917-4922.
- Cabi.W, Loper, Nieto; Blakeslea trispora genes for carotene synthesis; Applied Environment Microbiology Sept'04, Vol 70 pg. 5589-5594.
- Seethal, Rekha.s, Laxmi Ananth narayan ; use of metabolic stimulator and inhibitors for enhanced beta carotene and lycopene production by blakeslea trispora NRRL 2895 and NRRL 2896.;Bio resource Tech Vol.99, issue 8 pg: 3166-3173.
- Ralph.f, Anderson , Margie; Fwwd supplement microbiological production of beta carotene in shake flasks; J. agric food chem. 1958, Vol (7) PP: 543-545.
- Roukas Mantzouridou; production of beta carotene ; Applied Bio chain biotechnology 2001 Jan Pg.37-45.
- Httkp:hillagric.ac.in/edu/coa/horicuture/lecture/hort-351-lectures/hort-351 Lecture-32.pdf
- Synthesis of atypical cyclic and acyclic hydroxyl carotenoids in Escherichia coli transforms ants. J. Biotechnology 8:177-85 2.
- Rekha , Seethal ; media optimization for beta carotene production by Blakeslea trispora; bioresouce technology ,vol 99, issue 4, march 2008, pg 722-730
- Bhoua Wang, Lipinging lee, chin win; optimization of beta carotene production by newly isolated *Serretia marcescens;* electronic journal of biotechnology ISSN:0717-3458, doi: 10.2225,vol 15, issue 6
- Journal biotechnology and Bioprocess Engineering Volume 17, issue 6, PP1196-1204.
- Micro cell Fact. 2014; 13:100. Published online 2014 Nov 18 doi: 10.1188/s12934-014-0160-x.Pmcid: PMC7239400.
- Z.Naturforsch.58C, 562D567(2003); received December27.2002/February5.2003. Process biochemistry Vol42, issue2,Feb 07 pg282-293.
- Biochemical engineering journal Vol 67: Aug 12 Pg.203-207.

- http:hillagric.ac.in/edu/co/horticulture/lecture/hort-351-lecture-32
- P. Karnjanawipagul, W. Nittayanuntawech, P. Rojsanga and L. Suntornsuk ; Analysis of beta-Carotene in Carrot by Spectrophotometry ; Mahidol University Journal of Pharmaceutical Science 2010; 37 □ 1-2□ , 8-16
- Jung Hun Kim1 , Seon-Won Kim2 , Do Quynh Anh Nguyen1 , He Li1 , Sung Bae Kim1 , Yang-Gon Seo1 , Jae-Kyung Yang3 , In-Young Chung4 , Dae Hwan Kim5 , and Chang-Joon Kim Production of β-carotene by Recombinant Escherichia coli with Engineered Whole Mevalonate Pathway in Batch and Fed-batch Cultures Biotechnology and Bioprocess Engineering 2009, 14: 559-564 DOI/10.1007/s12257-008-0230-1
- R.Willstatter and W. Mieg .Justus liebigs Ann.chem 350,l,(1906)
- Meenu Thakur and Wamik Azmi An International Journal Nutraceutical beta-carotene from natural non-conventional sources and its applications . Annals of Phytomedicine 2(1): 59-73, 2013
- Sang-Hwal Yoon Gyeongsang ,Jae-Yean Kim Gyeongsang , Deok-Kun Oh KonkJay D Keasling Increased β-Carotene Production in Recombinant Escherichia coli Harboring an Engineered Isoprenoid Precursor Pathway with Mevalonate Addition Biotechnol. Prog. 2007, 23, 599−605
- Amitabha Das & Sang-Hwal Yoon & Sook-Hee Lee & Jae-Yean Kim & Deok-Kun Oh & Seon-Won Kim, An update on microbial carotenoid production: application of recent metabolic engineering tools Appl Microbiol Biotechnol (2007) 77:505–512 DOI 10.1007/s00253-007-1206-3
- Jens Høiriis Nielsen, John Villadsen, Gunnar LidénKluwer, Bioreaction engineering principles pg 254 Academic/Plenum Publishers, 2003 - Science - 528 pages
- Pauline M. Doran Bioprocess Engineering Principles , ISBN: 0122208552 Publisher: Elsevier Science & Technology Books Pub. Date: May 1995
- Evamaria Gruchattka[†], Oliver Hädicke, Steffen Klamt, Verena Schütz[†] and
- Oliver Kayser Email author Contributed equally, *n silico* profiling of *Escherichia coli* and *Saccharomyces cerevisiae* as terpenoid factories *Microbial Cell Factories* 201312:84 DOI: 10.1186/1475-2859-12-84
- Ioannis Voulgaris ,Evaluation of Options for Harvest of a Recombinant E. Coli Fermentation Producing a Domain Antibody Using Ultra Scale-Down Techniques and Pilot-Scale Verification Published online 00 Month 2016 in Wiley Online Library (wileyonlinelibrary.com) , DOI 10.1002/btpr.2220
- Lee PC, Mijts BN, Schmidt-Dannert C. pl Microbiol Biotechnol. Investigation of factors influencing production of the monocyclic carotenoid torulene in metabolically engineered Escherichia coli**.**2004 Oct;65(5):538-46. Epub 2004 May 27
- Carotenoid production by recombinant, c1-metabolizing methanothropic bacteria WO 2002018617 A2
- http://ajcn.nutrition.org/content/81/1/218S.long
- READER, V. 1925 Note on the lipochromes present in certain bacteria. Biochem. J., 19, 1039-1046.
- S.-K. Myung, Y. Kim , W. Ju , H. J. Choi & W. K. Bae ; Effects of antioxidant supplements on cancer prevention: meta-analysis of randomized controlled trials; Annals of Oncology 21: 166–179, 2010 doi:10.1093/annonc/mdp286 Published online 21 July 2009
- Dorogokupla AG. *Zdravookhr Kazakh* 1973;**10**:32–4 (cited in ref. **5**).
- Epstein JH. Effect of β-carotene on ultraviolet-induced cancer formation in the hairless mouse skin. *Photochem Photobiol* 1977;**25**:211–3.
- Van Poppel G, Goldbohm RA. Epidemiologic evidence for β-carotene and cancer prevention. *Am J Clin Nutr* 1995;**62**:1393S–402S
- BenSobin and Grant.L. Sthaley .The isolation and absorption spectrum maxima of bacterial carotenoid pigments,Applied biochem biotechnol November 13, 1941
- CHARGAFF, E., AND DIERYCK, J. 1932 Die Pigmente der Sarcina lutea. Naturwissenschaften, 20, 872-87
- CHARGAFF, E., AND LEDERER , E. 1935 Sur les pigments carotenoides de deux bacteria acido-resistantes. Ann. inst. Pasteur, 54, 383-388

- Bhosale P. and Gadre R. V. (2001), Production of beta-carotene by a Rhodotorula glutinis mutant in sea water Sudenko V., and Stelokova F. (1978),
- Anh Do Quynh Nguyen,Seon-Won Kim,Sung Bae Kim,Yang-Gon Seo,In-Young Chung,Dae HwaChang-Joon Kim Production of β-carotene and acetate in recombinant *Escherichia coli* with or without mevalonate pathway at different culture temperature or pH 04 January 2013
- Dias C, Sousa S, Caldeira J, Reis A, Lopes da Silva T New dual-stage pH control fed-batch cultivation strategy for the improvement of lipids and carotenoids production by the red yeast Rhodosporidium toruloides NCYC 921
- Martin et al., 1993a, b; Meyer and Du Preez, 1994; Buzzini and Martini, 1999; Bhosale and Gadre, 2001, Bon et al., 1997; Vijayalakshmi genera Rhodotorula, Rhodosporidium and Phaffia are known to form carotenoids from both synthetic and raw substrates for a long time
- Ben-Dore ,Steiner M, Gheber L, Danilenko M, Dubi N, Linnewiel K, Zick A, Sharoni Y,Levy J; Carotenoids activate the anti oxidant response element transcription system; Mol Cancer Ther. 2005 Jan;4(1):177-86.