ISSN No:-2456-2165

Study on Optimization and Pigment Analysis of Beetroot *(Beta-Vulgaris)* and their Application as Natural Dyes

R.Arthikha[1]
M Phil Research Scholar,
Department of Microbiology, Sri Ramakrishna College of Arts and Science for Women,Coimbatore
Tamilnadu, India

K.Madhanasundareswari[2]
Head of the Department,
Department of Microbiology, Sri Ramakrishna College of Arts and Science for Women,Coimbatore
Tamilnadu, India

Abstract:- The natural sources like fruits and vegetables are observed to have a rich source of nutrients, eco-friendly pigments and antioxidant etc. Along with it the peels of these sources which are regarded as waste are exploring the current environmental aspects on various applications. These natural colorant are efficiently processed by a simple aqueous extraction method. The analysis showed that the beetroot has a good source of betacyanin pigments that are found to be a water - soluble compound. The sample were subjected to various ranges of temperature, pH and other optimization study. It was kept in various range of temperature (RT - 110⁰), varied length of heating time (1- 10 min) and different range of pH (2 - 9). This was then further used to analyze and checked for its best obtained betacyanin content for its appropriate extraction and application. The study also enhances about the stability of the pigments by its best determined method. The analysis shows the best findings with a optimal conditions and stability for its future analytical applications. The aim of the study is to provide a efficient traditionally obtained natural dye that has various environmental friendly pigments and of higher values.

Keywords:- *Natural Source, Eco- Friendly, Pigment, Betalain, Betacyanin, Beta- Vulgaris, Traditional Colorant.*

I. INTRODUCTION

Pigments act as natural colorant which are observed to explore the world in all possible fields of lives. Many natural sources like leaves, flowers, fruits and vegetables also certain microorganism are found to be the principal producers of various pigmented compound. Among those synthetic pigments are also observed to be implemented in various fields of application, which is found to be harmful for environment and human health. However, natural pigments that are obtained from plants sources like chlorophyll, carotenoids, betacyanin etc., are used in medicines, preservation, food, textiles, molecular analysis that are probably safe towards the environment[1].

In most of the tropical and subtropical countries, an vast amount of vegetables are produced which are observed to be a major in commercial point of view. However, due to few adverse environmental reasons the product with higher nutrient value is left unused. *Beta vulgaris* commonly called as beetroot, which is mainly cultivated in India for its high nutrient value and yields about 31.25t/ha of roots. It is a herbaceous biennial plant with leafy stems of 1 - 2 meters tall and are found to be a edible taproots[2]. These roots are observed to be commonly in deep red - purple in color, also found with wide range of colors.

The most important fact about the higher plants is that they have a rich pigments range. Beets are readily observed to have excellent level of betalains pigments with explored activity towards major environmental and medicinal values. Betalains are water - soluble compound which contain nitrogen and don't show any reversible property like anthocyanins does for pH. Betalains are observed to have two variant category like betacyanins and betaxanthins, with an extract of red and yellow pigments respectively. Betacyanin is considered to be the major compound (95%) of red pigments in the sample extract[3]. The pigment is observed to explore with many application, Stable and are Eco-friendly.

In recent years use of chemical or synthetic dyes are extensively used in major fields, like food technology, agriculture, textile industry and also in molecular biology experiment. At present synthetic dyes is used in all industries that may poses higher health risk and also cause adverse environmental pollutions. The change of these hazardous chemicals must be done using natural colorant and which is best found in beetroots, the betacyanins are found to be more stable with good range of pH and temperature.

In our current research work the major objective is study the betalain pigments of beetroot at various range of pH, temperature and length of time to heat explore, which will help in best extraction of the compound from its sample. The extraction is observed with aqueous source because the betacyanin is water - soluble. This was also characterized for

its stability of the colorant towards light and this analysis is best carried out for its future applications in more field to develop a safe, cheap and healthy environment.

II. MATERIALS AND METHODS

A. Plant Material

Beetroot (*Beta vulgaris)* were brought from local super market, Sulur, Coimbatore. The vegetable sample were immediately processed to the laboratory for its further process. The fruit were thoroughly washed in water and was cut into small cubes. The cut cubes were treated with Benomyl 0.05% and air dried overnight under shade. The dried sample were finely grinded into a powder which was then passed through a fine mesh and packed in a clear polyethylene pouches. The sample were sealed and stored at 4º until further analysis.

B. Sample Measurements

The sample absorbance were carried out with a wavelength of 538nm using a spectrophotometer(Eppendorf) for the determination of betalain concentration whereas the pH was measured using pH meter (Vlab). The extracts were filtered thoroughly into the beaker using mira cloth and separated out the aliquot. The aliquots which was obtained as a result of each experimental process was allowed to cool before spectrophotometric measurement. All the experiments were processed in triplicates based with slight modification[4].

C. Confirmatory Test for Betacyanin

The betacyanin pigment in beetroot was confirmed using a simple confirmatory method . The sample were added with 2 M NaOH drop wise and change in the colour of the sample solution was observed.

D. Estimation of Total Betacyanin Content in Sample

The absorbance that was obtained as a result of each sets was used to calculate the total betalain concentration using the following formula [5].

$$\text{Pigment Content (mg L}^{-1}) = \frac{A \times MW \times 1000 \times DF}{\varepsilon \times \iota}$$

Where,
A = Absorbance of samples
MW = Molecular weight of pigment (betalain = 550 g/mol)
DF = Dilution factor
ε = Molar extinction coefficient of betalain (60.000 L M–1 cm–1)
ι = Path length (1 cm)

E. Determination of Water Volume to Extract Pigment

Five gram of powder were added to a beaker with 5, 10, 15, 25 and 30 ml of sterile distilled water for 10 min that determines which ratio of weight : volume yields the highest pigment range. The pH and absorbance were estimated. The best ratio range was carried out in next experiments.

F. Determination of Optimal Temperature of the Sample

Five gram of beetroot powder was added into a beaker with 10 ml of distilled water and dye was extracted at room temperature (RT) for 10 min. The absorbance and pH of the obtained aliquots were measured. The same sets of experiment was also carried 50, 60, 70, 80, 90, 100 and 110ºC. Among these the best obtained results were carried out in the next process.

G. Determination of Optimal Length of Time to Heat Exposure

Five gram of the vegetable powder was immersed in 10 ml of boiling distilled water. The processed solution was left for 1 min. The pH and absorbance of the aliquots of the sample was measured. The experimental sets were repeated at 2, 3, 4, 5, 6, 7, 8, 9 and 10 min maintaining a constant temperature. The best length of time was carried for further process.

H. Determination of Optimal pH Range

Five gram of sample powder was dissolved in 10 ml of distilled water. This sample was separately taken in a test tubes. Each test tubes were labeled and alerted the pH range (2, 3, 4, 5 and 6) by adding 1M citric acid. All the tubes were adjusted with appropriate labeled pH and were heated at 100º. The process of extraction was done for 10 min by maintaining a constant temperature for all the samples. The pH and absorbance of the extract was determined. The best range was subsequently carried out for next experiments.

I. Determination of Colour Stability of the Sample

Five gram of powder was added to 10 ml of pH 4 sample and heated to 100ºC. The extraction process was done for 10 min by maintaining the same temperature. The pH and absorbance was checked for the aliquots. Both the aliquots(Dark and Light) were kept in RT, 4º and - 20º. The samples were determined in both dark (wrapped using aluminum foil) and light storage for 7 days.

III. RESULTS AND DISCUSSION

A. Confirmatory Test for Betacyanin

The sample were added with 2 M NaOH drop wise and the colour changed to yellow, which indicates the presence of betacyanin**.** This refers to the results of [6] explaining that the compound in the sample is betacyanin pigment. Results are shown in figure 1.

ISSN No:-2456-2165

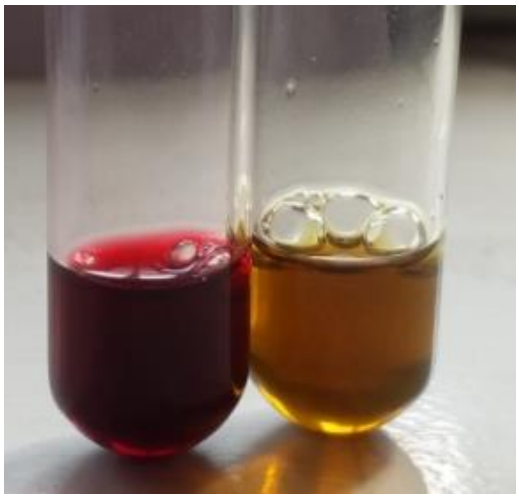

Fig 1:- Presence of betacyanin (Yellow color)

B. Determination of Water Volume to Extract Pigment

The pH for five gram of powder extracted with different volumes of sterile distilled water did not show any significant variation. Whereas in Figure 2 shows the absorbance values of the total betalain content obtained from five gram of powder in 5, 10, 15, 25 and 30 ml of water was 9.08, 12.69, 12.04, 11.13 and 10.26 mg L^{-1} , respectively. This shows that the highest yield was obtained in sample with five gram in 10ml of distilled water.

It is possibly determined that the extraction process is best obtained at a higher yield of betalain pigment from beetroot powder is by using the ratio 1:2 (weight : volume). Similarly, [4] found the extraction of the particular compound using Dragon fruit.

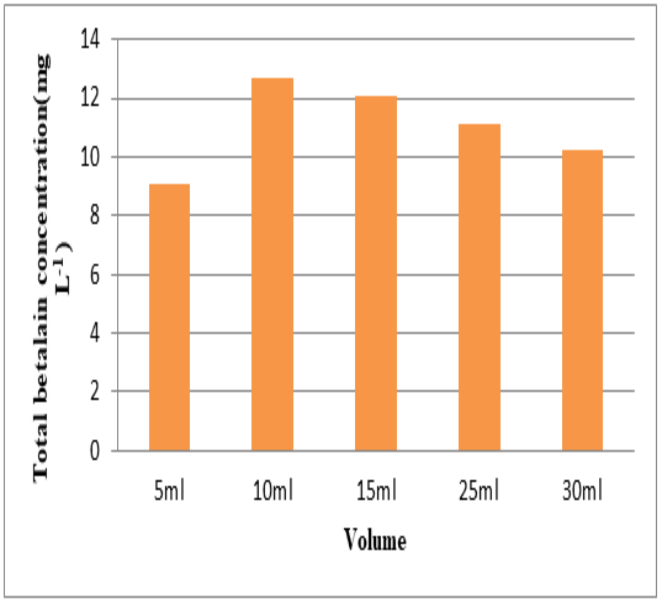

Fig 2:- Total betalain concentration of 5g powder in different volumes of water

C. Determination of optimal temperature of the sample

The figure 3 shows the absorbance level of total betacyanin content obtained from five gram of powdered sample in 10ml of distilled water which was carried out at different temperature at RT, 50, 60, 70, 80, 90, 100 and 110ºC were 5.23, 10.02, 11.87, 14.34, 17.79,18.50, 24.74 and 19.54 mg L^{-1} respectively. This shows that the highest yield of the betacyanin was obtained at 100 °. There was no significant change the pH value of the heated samples.

As shown in figure 3 it associate that the best yield is extracted at 100ºC which despite exposure even at extreme heat, it is found that the betalain can regenerate by recondensation of the hydrolysis states that he product can also regain its colour[9].

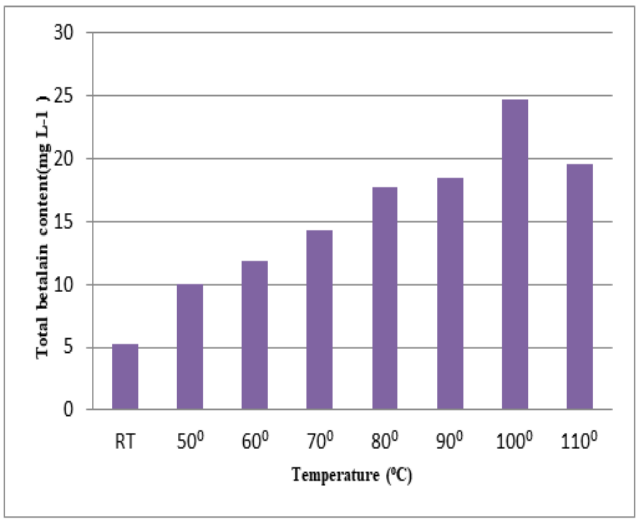

Fig 3:- Total betalain content in different temperature

D. Determination of optimal length of time to heat exposure

The results of heat exposure is shown in figure 4 which states that the total betalain concentration observed from the extract of five gram of powder in 10ml of sterile distilled water at 100ºC at different length of time were found to have a significant difference. The optimal length time used to analysis was 2, 3, 4, 5, 6, 7, 8, 9 and 10 min were 5.32, 6.35, 10.55, 10.54, 13.29, 13.69, 13.28, 14.52 and 16.38 mg L^{-1} respectively. The pH dint not show any significant change with this beetroot sample.

The results from the figure 4 represents that the sample will be best extracted at 100ºC at time length of 10 min. This depicts that the betalain can be obtained high yield even at 10 min. Therefore, it shows that betalain content can be obtained at a high length time of exposure. Beyond it 10 minutes it was found to lower the extraction content of betalain production[5].

ISSN No:-2456-2165

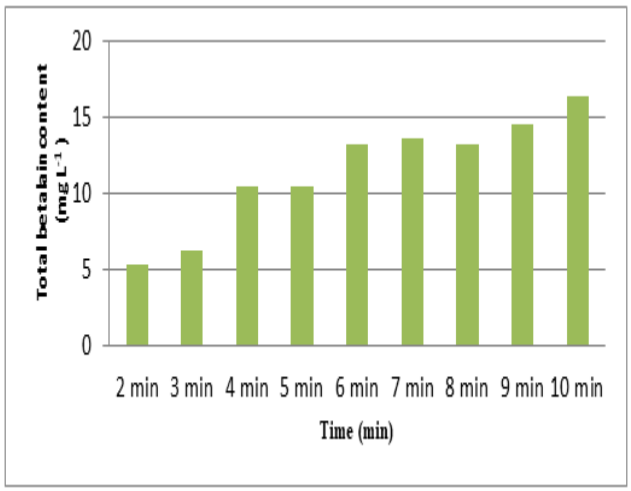

Fig 4:- Total betalain content in different length of time to heat exposure

E. Determination of Optimal pH Range

Figure 5 represents that the total betalain content extracted from five gram of powder in 10 ml distilled water at 100ºC for 10min in varied range of pH obtained different concentration range. The pH range was from 2, 3, 4, 5 and 6 of sample were 10.09, 12.36, 17.25, 15.24 and 13.58 mg L^{-1} respectively. The best highest yields of betalain was found in pH 4 of the sample.

This determination showed that the best extraction is taken at pH 4 with the highest level of betalain content by carrying out the process of obtained results. As per the previous studies, it shows that betalain pigment favors pH range from pH4 to pH6 in both aerobic and anaerobic condition[5]

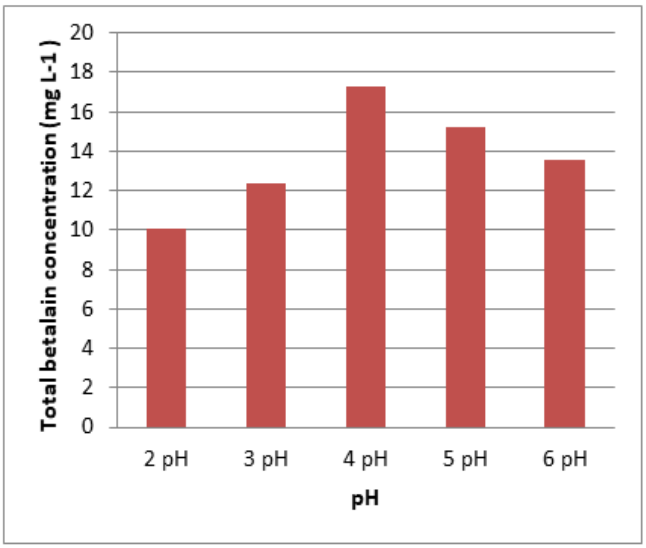

Fig 5:- Total betalain content at different pH

F. Determination of Colour Stability of the Sample

The figure 6 shows that stability of the extracted pigment with five gram in 10ml of distilled water at 100º for 10 min showed a high yield differences. The betalain concentration for both sample and control was determined over a period of 7 days at RT, 4º and -20ºC. The pH did not exhibit any significant change during the incubation period. Whereas the total betalain content showed a higher yield when it was stored at 4ºC in the dark, from 23.35 mg L^{-1} in 0^{th} day to 37.25 mg L^{-1} in 7^{th} day. The other storage temperature was observed to produce a decrease in the concentration.

The results shows a clear picture that the stability of the pigment is high in case of dark storage at 4ºC. Thus, proves that they do not show any degradation activity during dark incubation. Whereas in prolonged light storage the content got decreased because betalain is light - sensitive and may degrade in the absorption of visible light and ultra - violet range [9].

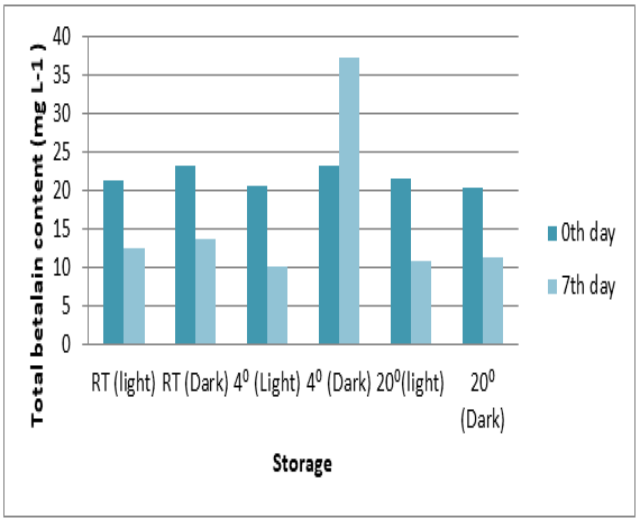

Fig 6:- Total betalain content at different storage temperature

IV. CONCLUSION

The beetroot is found to be a avenue as a major source of natural pigments with high content of betacyanins. The study shows that the beetroot sample yields a highest extraction with 5gram in 10ml of distilled at 100ºC in 10min with a pH range of 4. The highest temperature range of betalain explores that they can with stand highest degree of temperature by not changing its character. Also the pH of the sample shows the retention capacity to tolerate higher level acidic nature. The stability of the pigments is also the best part of this betalain that significantly shows a drastic increase in its activity. Thus, with all this promising findings so far states that the powder of beetroot has a high content of betacyanin which a water - soluble content and can have a excellent application on textile, medicines , preservatives and molecular analysis. This work takes a step towards the

replacement of the synthetic dyes with the natural dye which is eco-friendly and safe.

ACKNOWLEDGMENT

The authors would like to thank the Management, the Principal of Sri Ramakrishna College of Arts and Science for Women, Coimbatore for their kind support towards completing this work.

REFERENCES

[1]. DELGADO-VARGAS, F., JIMÉNEZ, A. R. AND PAREDES-LÓPEZ, O.2000.'Natural Pigments: Carotenoids, Anthocyanins, and Betalains — Characteristics, Biosynthesis, Processing, and Stability', *Critical Reviews in Food Science and Nutrition*, 40: 3, 173 — 289 DOI: 10.1080/10408690091189257 URL: http://dx.doi.org/10.1080/10408690091189257.

[2]. STRACK D, VOGT T, SCHLIEMANN W 2013. Recent advances in betalain research,*Phytochemistry*, 62(3): 247–69 https://doi.org/10.1016/s0031-9422(02)00564-2.

[3]. CAI, Y., SUN, M., SCHLIEMANN, W., & CORKE, H. 2001. Chemical stability and colourant properties of betaxanthin pigments from Celosia argentea .*Journal of Agricultural and Food Chemistry*, 49(9), 4429–4435 https://doi.org/10.1021/jf0104735

[4]. K.V.HARIVARINDARAN, REBECCA O.P.S AND CHANDRAN. S, 2008. Study of Optimum Temperature, pH and Stability of Dragon fruit Peel for use as Potential Natural Colorant, *Pakistan Journal of Biological Science*, **11(18)**,2259-2263 https://doi.org/10.3923/pjbs.2008.2259.2263

[5]. HERBACH, K.M., C. MAIER, F.C. STINTZING AND R. CARLE, 2007. Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice. *European Food Research and Technology* 224: 649-658 https://doi.org/10.1007/s00217-006-0354-5 .

[6]. P. SUGANYADEVI, M. SARAVANAKUMAR, K.M. ARAVINTHAN, A. ARUNKUMAR, R.K. KRISHNA AND S. KARTHIKEYANI,2010 Extraction of Betacyanin from Red Beet root (Beta vulgaris L.) and to evaluate its antioxidant potential, *Pharm. Res.*, 3, 2693.

[7]. STRACK D, VOGT T, SCHLIEMANN W 2013. Recent advances in betalain research,*Phytochemistry*, 62(3): 247–69 https://doi.org/10.1016/s0031-9422(02)00564-2

[8]. STINTZING F.C AND R.CARLE, 2007. Betalain-emerging prospect for food scientist, *Trends Food Science and Technology*, 18: 514-525 https://doi.org/10.1016/j.tifs.2007.04.012 .

[9]. AZEREDO HMC**.** 2009, Betalains: properties, sources, applications, and stability – A Review. *International Journal of Food Science and Technology*, 44(12), 2365–2376 https://doi.org/10.1111/j.1365-2621.2007.01668.x .