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Optimizaton of extraction of betalain pigments from beta vulgaris peels by microwave pretreatment

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Abstract. The effect of microwave assissted extraction of pigments from beetroot peels was studied. Oven Drying followed by microwave assisted extraction was decided as the experimental procedure to be followed because of better absorbance in the range of 0.2-0.4. A microwave assisted extraction procedure is said to open the vacuolar pores inside beetroot powder.The Box-Behnken method was used as the RSM optimization technique. A quadratic model was suggested for both the solvents used. The optimized conditions for Solvent A were pH 5.20, Microwave Power of 224.61MW and Time 57.06 seconds for a Betanin Concentration of 229.264mg/L. The optimized conditions for Solvent B were found to be pH4.74, Microwave Power 384.25MW, Time 74.91 seconds and Betanin Concentration 472.113mg/L. The order of the extraction process was calculated as 1.42 and the rate constant as 0.00126. FTIR results confirm similar functional groups before and after the treatment. FTIR results confirm the presence of O–H stretch, C-H stretch, H–bonded, C-C stretch, N–H bend.

1. Introduction

Waste management is colossal environmental problem on a day-to-day basis. Waste generated from processed fruits and vegetables are mostly used as a source of bioactive compounds viz., tocopherols, polyphenols, carotenoids. These phytochemicals may be used as natural antioxidants and as functional food ingredients to replace their synthetic equivalents [1-4]. Beetroot is one of the most potent vegetable because of presence of significant amounts of phenolic acids that are highly antioxidants [5, 6]. It was established that beetroot pomace extract has a high content of bioactive compounds, namely phenolics and betalains, and possesses important antioxidant and anti-proliferative activities [7-9]. Additionally, beetroot (Beta Vulgaris) is the chief source of natural red dye. The pigment which causes the red colour is called betalain which falls under the major category of betalains. Betalain, extracted from beetroot, is also called beetroot red and is chemically a glucoside and hydrolyses into sugar glucose and betanidin. [10-12]. Betalains are alkaloid pigments that are found in some families of plants belonging to the order Caryophyllales, but in no other plants. Some fungi e.g. Fly Agaric are also found to have these types of pigments.

There is a renewed interest in the food industry in betalains as they were identified as antioxidants by in vitro methods. It is widely used as a food colorant in food products like yogurts, ice-creams, sweets and other products. Though it is both cheap and has no known allergic side-effects can only be used in ice-cream, sweets and other confectionary because of its sensitivity to temperature changes i.e., it discolours when heated. Variation of pH also affects the colour of the pigment and it is reddish violet in acidic conditions. Beetroot Vacuoles are store houses of pigments hence are used as

markers by scientists who wish to extract intact vacuoles from plants for investigation. The extraction of the pigments can be effected only by the disruption of the membranes. This is possible by the application of detergents, solvents, heat shock, etc.
The most preferred method to produce colour concentrates is thermal treatment; but betalain is highly sensitive to heat so an alternate method may be suitable with lesser time of exposure to heat [11, 13]. A change in colour intensity and anti-oxidant activity may result [13]. During extraction process, heating the plant tissues with solvents by exposing them to microwaves that increases the kinetic of extraction, is called microwave-assisted extraction (MAE). A number of advantages is attributed to MAE over traditional method of extraction for e.g., higher extraction rate associated with shorter extraction time, less solvent to express more compounds from complex matrices of vacuoles, especially natural products. The application of MAE for extraction of natural products gained momentum in the late 1980s. Due to rapid technological developments, MAE has become one of the cost-effective extraction methods available today. Various parameters such as pH, microwave power and microwave time were used for the extraction process.

Extraction is of pigment is normally done for whole tuber but not the peels. In order to reduce wastage and value addition the peels are subjected to different extraction conditions and red pigments are extracted. The study focused on: 1) the best method of drying the peels before it can be used, 2) selecting solvents for pigment extraction from the dried sample, 3) optimizing operating parameters using Response Surface methodology and 4) estimation of kinetics of the extraction process.

2. Materials and methods

2.1 Materials

Beetroot peels collected from our university mess was oven dried at 55˚C for 15-18 hrs and ground to make a powder of 80 mesh size. Solvents used for extraction were citric acid crystals L.R (Nice Chemicals Pvt. Ltd., India) and ethanol (99.9%).

2.2 Extraction and concentration of betalain pigment

The ground dry beetroot peel powder (1gm) was mixed with water (5ml) and made into a paste. This was subjected to microwave irradiation followed by extraction for 10 min. The extracted solution was centrifuged for 5 min at 2500 rpm in a cooling centrifuge.

2.3 Determination of Betalain content

The amount of betalain in each case was calculated as mg/g basis. The concentrated red pigments solution was diluted with distilled water and the extinction measured at wavelength 535nm. The quantification was expressed as mg betalains/ 100g powder using the following equation as determined by Castellar et al., [2003] [14].

$$Total\ betalain\ content\ \frac{mg}{g} = A \times DF \times MW \times 1000/\in L \quad (1)$$

Where:
A: Absorption value at 535 nm, DF: Dilution volume, L: Path length of cuvette 1 cm,
MW: Molecular weight of betalain (550 g/mol), ϵ: The extinction coefficient for betalain 60000 l/mol.
The betalain contents were determined for samples treated with various conditions of extraction parameters namely solvent (citric acid and ethanol), microwave power (180, 300 and 450W), microwave time (30, 60 and 90s), pH (4, 5 and 6) for citric acid solvent, and concentration (25, 50 and 75%) for ethanol.

2.4 Response Surface Methods for Process Optimization

Response surface methodology (RSM) is a statistical tool to explore the relationships between several variables and one or more response variables. Design-Expert® software was used for response surface methodology (RSM). It enables the user to find optimal operating conditions , the "sweet spot"

where all specifications are met by generation of 3D and contour maps and multiple responses can be optimized. Thus Response Surface Methods (RSM) can lead the user to the peak of process performance.

Factorial designs are applied for fitting second-order regression models. If a first-order model suffers lack of fit, a second-order model can significantly improve the optimization process considerably by allowing for interaction between variables and surface curvature. A general second-order model is defined as

$$y_0 = a_0 + \sum_{i=1}^{n} a_i x_i + \sum_{i=1}^{n} a_{ii} x_i^2 + \sum_{i}^{n} \sum_{j=1}^{n} a_{ij} x_i x_j \quad (2)$$

The building of a quadratic response surface model for n variables requires a three level study, so that the modifying parameters can be estimated. Therefore, for a response surface methodology at least (n+1) (n+2) / 2 function evaluations are essential. For a three level full factorial the number of experiments grows exponentially by 3n and becomes impractical. A full factorial design typically is suggested for five or fewer variables.

Generally, Central Composite Design [15,16] with α value is ideal for RSM but the Box-Behnken design is considered as an efficient alternate option. It has three levels per factor, but does not consider the corners of the space and fills in the combinations of center and extreme levels. It combines a fractional factorial with incomplete block design to avoid the extreme vertices and present an almost rotatable design with three levels per factor [16].

The range of design variables selected for the investigation is given in Table 1.

Table 1 Range of design variables

Variable	-1	0	+1
pH (x_1)	4	5	6
Microwave power MW (x_2)	180	300	450
Time in s (Solvent A) (x_3)	30	60	90
Time in s (Solvent B) $(x_{3)}$	25	50	75

2.5 Fourier Transform Infrared Analysis (FT-IR)

Fourier transform infrared spectroscopy (FTIR) is a technique that is used to obtain an spectrum of absorption, emission, photoconductivity by energy transmission in infrared region or Raman scattering of a solid, liquid or gas. Solid samples were used to identify organic and in some cases inorganic materials using changes in the functional groups. The FT-IR spectra of the samples were recorded in transmission mode using Perkin Elmer spectrometer by placing the sample in KBr windows.

2.6 Kinetic Study

In the absence any chemical reaction it may be assumed that a pseudo-order kinetic model will suitably describe the mass-transfer process like extraction, adsorption etc. In general, the mass transfer rate of the extraction process may be controlled by kinetic, diffusion or mixed process. To decide the extraction rate it is reported as necessary to understand the experimental information to fully describe the kinetics. For extraction kinetics, one criterion generally used to identify the extraction regime is independence of the extraction rate on the stirring speed in constant interfacial area cell, so the effect of chemical reactions proceeds only to a small extent. [17].

A pseudo I order and empirical form of extraction rate equation can be written as:

$$\frac{dY}{dt} = kY \; or \; Y = kY \quad (3)$$

and $$Y = kC^n \quad (4)$$

where Y is the concentration of extract and k- reaction constant for I order reaction and 'n' is the empirical Order of Reaction. Kinetic modeling studies were done by conducting experiments at the optimum values which was obtained from the statistical analysis.

3. Results and Discussion

3.1 Optimization

Optimization studies were carried out with the experimental results using Response Surface Methodology (RSM). The 3 factor Box Behnken Design was chosen to find the optimum.

Table 2 Box-Behnken Design for the pigment extraction

Solvent A – Citric Acid				Solvent B - Ethanol			
pH	MW	Time s	Betalain content mg/L	pH	MW	Time s	Betalain content mg/L
6.0	300	90	163.63	4.0	150	50	234.44
5.0	300	60	241.31	5.0	450	75	459.24
4.0	300	30	194.56	5.0	150	75	432.44
6.0	150	60	156.76	5.0	450	25	308.01
4.0	450	60	220.69	6.0	300	75	407.10
4.0	300	90	190.44	4.0	450	50	276.38
5.0	300	60	241.38	6.0	300	25	297.01
5.0	300	60	242.32	5.0	300	50	418.02
5.0	150	90	198.02	4.0	300	75	381.56
5.0	450	30	206.94	5.0	150	25	267.54
5.0	300	60	241.31	6.0	150	50	274.31
5.0	150	30	204.88	6.0	450	50	318.31
6.0	450	60	159.50	4.0	300	25	259.88
5.0	300	60	242.41	5.0	300	75	397.63
4.0	150	60	181.50	4.0	150	50	234.67
6.0	300	30	121.15	6.0	300	50	319.82
5.0	450	90	203.50	5.0	450	25	307.23

The results showed that, Quadratic model was significant after ANOVA and gave a final regression equation for obtaining betalain concentration at any given parameters namely pH, microwave power, concentration and microwave time. The model with the least standard deviation, R-Squared values and Adjusted R-squared values close to 1 and lowest PRESS values is suggested to be good fit for the data. A quadratic model with R-square values of 0.9663 and 0.9858 was hence chosen from the ANOVA (Table 3). With this quadratic model, optimum condition for extraction using each solvent for maximum yield of betalain was estimated [18].

Table 3 Model Summary Statistics

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.040	0.1389	-0.0598	-0.3198	0.032	
2FI	0.045	0.1432	-0.3709	-1.3954	0.058	
Quadratic	0.010	0.9683	0.9276	0.4933	0.012	Suggested
Cubic	0.000	1.0000	1.0000		+	Aliased

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared
Linear	53.71	0.6240	0.4986
2FI	65.67	0.6252	0.2503
Quadratic	18.10	0.9858	0.9430
Cubic			

The second-order models illustrate quadratic surfaces such as minimum, maximum, ridge, and saddle. A stationary point indicates the existence of an optimum. The stationary point is the permutation of design variables where the surface may be at a maximum or a minimum in all directions [19]. This point is determined using matrix algebra (Table 4).

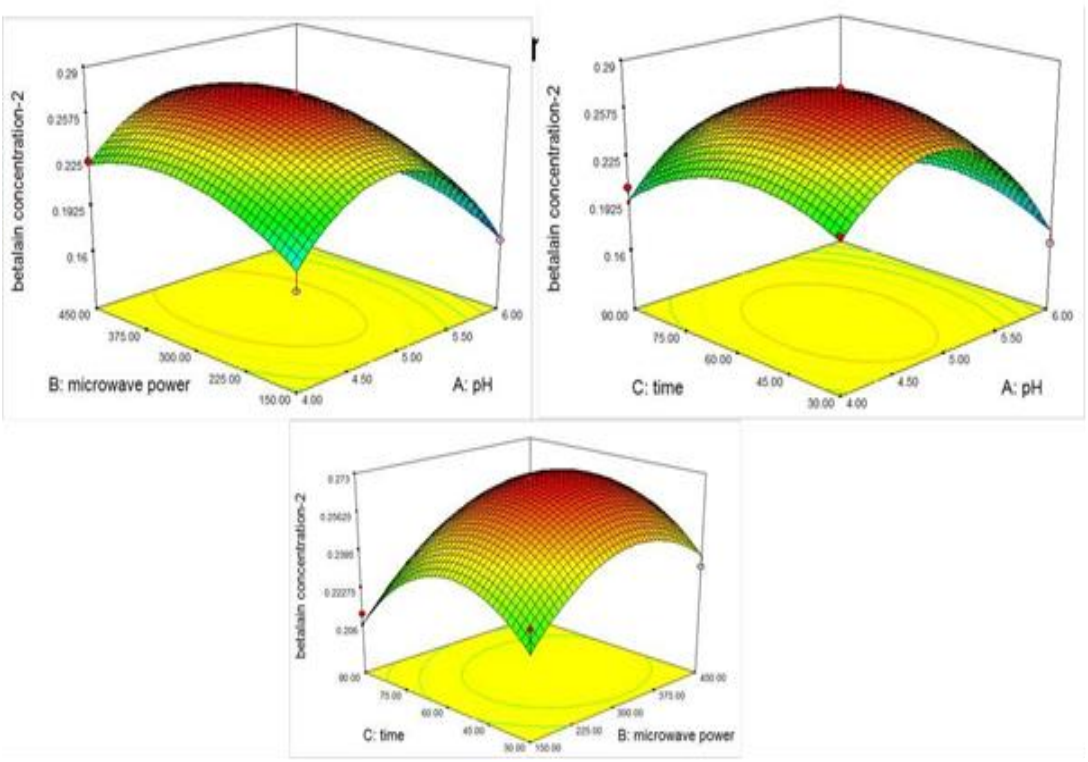

Figure 1 RSM for Solvent A

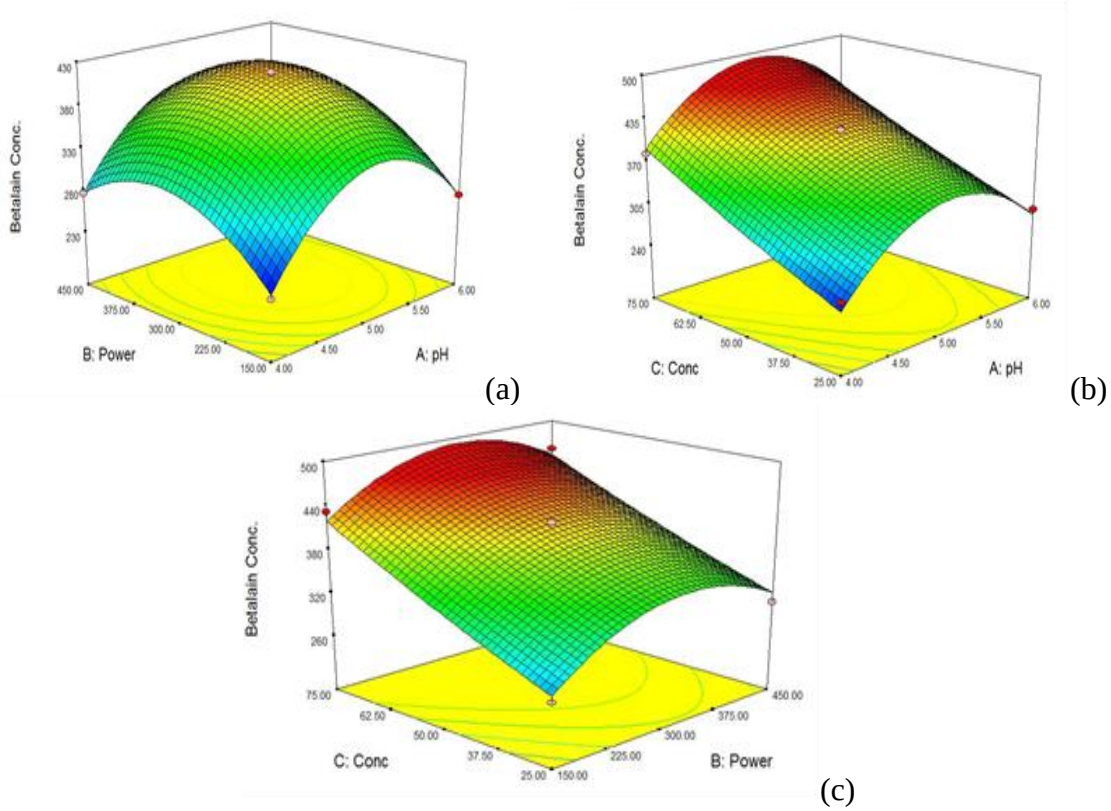

Figure 2. RSM for Solvent B

The regression equation for the Solvent A and Solvent B were given as,

$$y = -992.22 + 425.34x_1 + 0.844x_2 + 2.503x_3 - 0.067x_1x_2 - 0.029x_1x_3 + -42.543x_1^2 - 0.021x_3^2 \quad (5)$$

$$y = -2247.22 + 885.670x_1 + 1.646x_2 + 2.857x_3 + 3.438x_1x_2 - 0.117x_1x_3 + -86.281x_1^2 \quad (6)$$

From the above equations it was observed that pH is most significant parameter in the extraction process. The interaction of pH and microwave effected the extraction positively. Desirability was found to be 0.75 in case of citric acid solvent and 1 in case of ethanol solvent, indicating that ethanol [20-21] is a better solvent for extracting betalains from beetroot peels.

Table 4 Optimized values of parameters for the solvents

Parameter	pH	Microwave power	Microwave time	Betanin Concentration
Solvent A Citric acid	5.2	224.61MW	57.06 sec	229.264mg/l
Solvent B Ethanol	4.74	384.25MW	74.91s	472.113mg/l

3.2 Extraction Kinetics

The kinetics of extraction of betalain from beetroot peels in the presence of ethanol is a diffusion-controlled process occurring at the interface as evident from a pseudo I Order kinetics. When analysed for a general empirical model also the studies indicated that the empirical order of extraction process was found to be 1.42 that is nearer to I order with a rate constant 0.00126 (Fig 3). Sturzoiu et al 2011 [22], studied a two stage kinetic modelling for extraction In that solid liquid extraction is concerned, the initial period of extraction is very fast. The proposed model had a first order kinetic term as estimated in the present study.

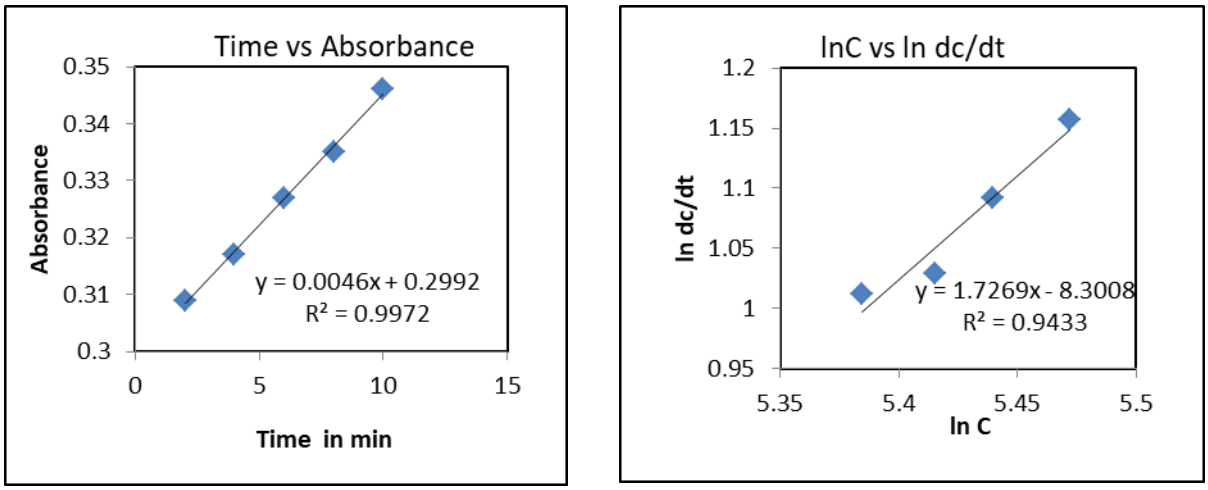

Figure 3 Kinetics for extraction of Betalain

3.3 FT-IR characterization

The FTIR images (Fig.4) shows major peaks at the following wave numbers and the corresponding bonds are given in Table 4. In FTIR images the common peaks at 3442.94 cm-1, 3134.33cm-1, 641.42 cm-1 and 1400.32 cm-1corresponding to O-H, C-H, N-H for 1oamines and C-C stretch respectively were observed. There was a minor or no shift with varying absorbance intensity for all the samples analysed. In samples dried at higher microwave output powers of 180 and 300 W additional peak at 821 cm-1 was observed, indicating a better yield of extractible suggesting higher power of microwave energy opened more pores for improved dehydration.

Table 4 FT-IR peaks and bonds

Frequency, cm^{-1}	Bond	Functional group
3442.94	O–H stretch, H–bonded	Alcohols, Phenols
3134.33	C-H stretch	Aromatic
1400.32	C-C stretch	Aromatic
1641.42	N–H bend	1° amines

From the Fig 4 a similar functional groups before and after treatment were observed.. This shows that the treatment does not play a major role in changing the chemical structure of the extract. It might be undesirable if the pigment structure is altered due to microwave treatment thus making it unsuitable pre-treatment for extraction.

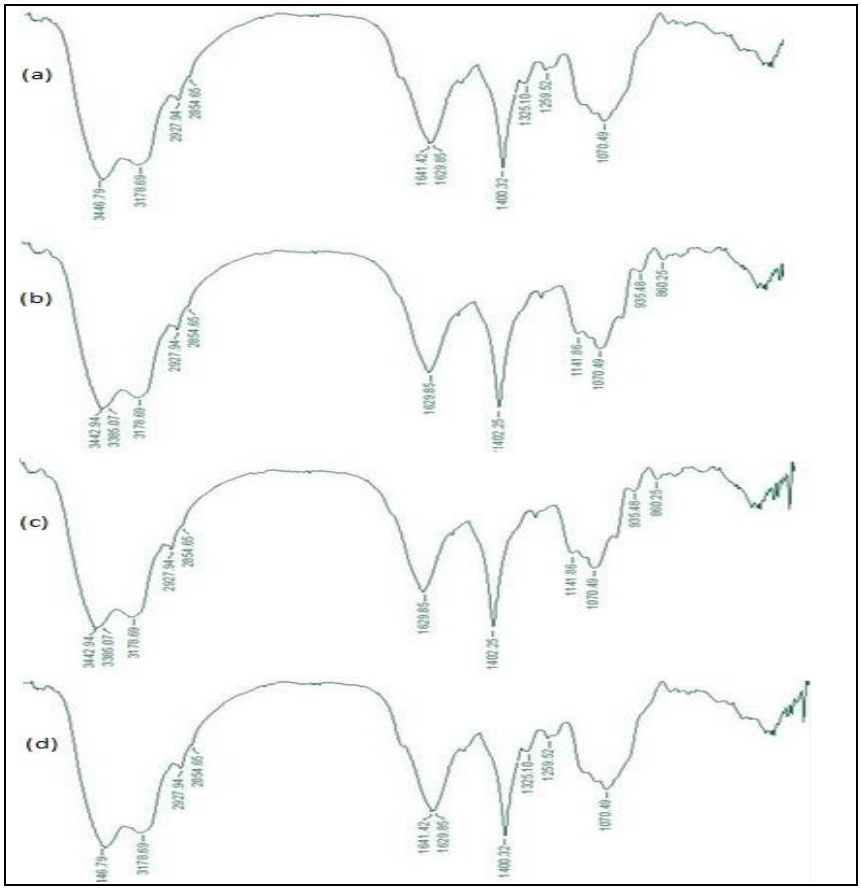

Figure 4. FT-IR spectrum of pigments (a) Untreated peels, (b) 450W(c) 300W MW power (d) 180W MW power

4. Conclusion

Oven Drying followed by microwave assisted extraction was decided as the experimental procedure to be followed because it gave better extraction concentration of mg/g. Microwave assisted extraction procedure is said to open the vacuolar pores inside beetroot powder and gave enhanced extraction. The optimization using the Box-Behnken method suggested a quadratic model with optimum conditions for process parameters. A pH of 5.20, 224.61MW Microwave Power and Time of 57.06 seconds for Betalain Concentration of 229.264mg/L was optimum for citric acid. Similarly, for ethanol pH of 4.74, 384.25MW Microwave Power and Time of 74.91 seconds for Betalain Concentration of 472.113mg/L was found optimum. A pseudo order of the Extraction process was calculated as 1.42 for ethanol as solvent. The FTIR results confirmed that the treatment does not play a major role in changing the chemical structure of the pigment extracted.

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