

Comment [RM1]: Manuscripts for reviewers in ms word format not pdf

**Different methods of betalain extraction from red beetroot (*Beta vulgaris* L.) for preparation of solid food colour and beetroot leather.**

## Abstract

An experiment was conducted at the Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh during the year 2021-2022. This experiment was conducted to assess the best extraction method for highest concentrated Betalain formulation. The best betalain extraction method was obtained from freezing – ground and squeezing method, which had highest betalain extract (530ml/kg fresh beetroot) with pH 5.08. Altogether 11 treatments were taken for both betalain powder and beetroot leather preparation. Combination of Freezed-Betalain and 3gm corn starch (T<sub>9</sub>) was found best for betalain powder preparation in terms of physio-chemical properties, pH and organoleptic test viz. color and appearance, texture and taste. The combination of beetroot pulp 860gm + 1gm salt + 1gm citric acid + 200gm sugar + 4gm maltodextrin powder was best for preparation of beetroot leather in terms of physio-chemical properties and organoleptic taste. The powder and leather samples were stored both at ambient temperature (28 ± 5 °C) and refrigerated condition (5 ± 2 °C) for 60 days. Beetroot leather performed well under cold temperature than the room temperature and on the other, betalain powder performed better in room temperature. The maximum Benefit cost ratio (2.46) was found in T<sub>9</sub> (Freezed Betalain + 3gm corn starch).

Comment [RM2]: More clearly the novelty of this research

**Keywords:** Betalain extraction , Beetroot powder , Beetroot leather, storage ,physiochemical properties .

## Introduction

Fruits and vegetables have historically held a place in dietary guidance because of their concentrations of vitamins, especially vitamin A and C; minerals, especially electrolytes; and more recently phytochemicals especially antioxidant. India is world's second largest fruit and vegetable producer, produced around 81.285 million tons fruits and 162.187 million tons of vegetables which accounts for nearly 14.0% of country's share in the world production of vegetables in the year 2012. It ranked amongst the world's five largest producers of over 80% agricultural produce items, encounters a waste of close to 25% worth of produce (NHB, 2013). Most of the fruits and vegetables produced in India are still consumed fresh except for a very small quantity going into the manufacturing of pickles, fruit and vegetable drinks, tomato ketchup, fruit jelly, candy, juices, leather, and dried and fried fruits. Due to the perishable nature of the fruits and vegetables they require immediate processing to avoid post-harvest losses. As per National Institute of Nutrition (NIN, 2004), nutritional composition of beetroot constituted Moisture (87.7g), Protein (1.7g), Fat (0.7g), Mineral (0.8g), Crude fibre (0.9g), Carbohydrates (8.8g), Calories (43Kcal), Calcium (18.3 mg/100g), Phosphorus (55 mg/100g) and Iron (1.19 mg). (Gopalan *et. al.*, 2004)

## Material and Methods

An experiment on “**Different extraction methods of Betalain from red beetroot (*Beta vulgaris* L.) for preparation of solid food colourant and Beetroot Leather**” was laid out in design CRD. The experiment was conducted at post-harvest laboratory of Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh during 2021-22.

## Betalain extraction

Betalain extracts were prepared from 1 kg of fresh beetroots for each method. First beetroots were peeled, ground, and squeezed and betalain was collected in beaker.

In the 1st step ground 850gm/1kg (after peeling) beetroots and extracted betalain by squeezing through muslin cloth manually, 510ml betalain was collected in a beaker, in 2nd

step beetroots 1kg beetroot peeled 820gm root (after peeling) were cut in to small pieces of 1/8' inch and boiled with water then for 30 min , boiled roots were again ground and betalain was extracted manually by squeezing in muslin cloth 290ml betalain collected, in 3rd step 845gm beetroots were cut in to small pieces and freezed at -20°C in a commercial freezer for 12-14hr, after freezing beetroots were kept in normal temperature for 30-45 min and again grounded and extracted betalain through muslin cloth, highest amount betalain collected were 530ml with dark colour.

### **Preparation of betalain powder**

After extraction of betalain by following above 3 steps extracted betalains were divided equally into 9 aluminium foils, (each treatments to 3 aluminium foils) then 1g, 2g, and 3g of organic corn starch were added accordingly on 9 samples to prepare a powder form by drying in Tray dehydrator. At 50-55 degree c temperature sample were dried for 14-18 hr then powdered betalain collected.

Image 1: **Preparation of beetroot leather**

### **Moisture**

The initial moisture content of leather was determined by standard oven method (**Ranganna, 1986**). The sample was dried in oven at 55 °C temperature until the material became completely dry. Then sample were removed from oven and cooled in desiccators for 10 min. Then the weight of the dry sample was taken. The per cent moisture content was calculated by using equation.

$$M = \frac{W1 - W2}{W1} \times 100$$

Where,

M = Moisture content

W1 = Weight of wet sample, (g)

W2 = Weight of dry sample, (g).

Knowing the initial moisture content of sample, its dry weight was calculated and then the reduction in moisture content with respect to drying time was determined using mass balance.

### Betalain

The total betalain pigment was determined by the methods reported by **Stintzing *et al.*, (2003)**.

### Reagents

1. 0.2 M Stock solution of sodium phosphate dibasic: Prepared by dissolving 28.38 g of sodium phosphate dibasic in distilled water and make up volume 1 liter.
2. 0.1 M Stock solution of citric acid: Prepared by dissolving 19.21 g of citric acid in distilled water and make up volume 1 liter.
3. McIlvaine buffer: Prepared by mixing 0.1 M citric acid (29.65 ml) and 0.2 M sodium phosphate dibasic (70.35 ml).

### Statistical Analysis

The data recorded during the course of experimental investigation were subjected to statistical analysis of "Analysis of variance" technique (**Fisher and Yates, 1967**) for drawing conclusion. The significance and non-significance of the treatments were judged with the help of 'F' (Variance ratio) test the significant differences between the means were tested with the critical differences at 5% probability level.

## Result and Discussion

### Compositions of betalain powder /100gm

Table No. 1.

#### Physico-chemical properties of betalain powder

Parameters	Contents	
Energy	338.83	Kcal/100gm
Carbohydrate	75.94	g/100g
Protein	18.52	g/100g
Fat	0.11	g/100g
Ash	2.34	g/100g
Moisture	13.09	g/100g
Crude fibre	1.59	g/100g

Table 2:

### Compositions of beetroot leather

Parameters	Contents	
Energy	350.82	Kcal/100gm
Carbohydrate	72.71	g/100g

Protein	12.52	g/100g
Fat	1.10	g/100g
Ash	7.73	g/100g
Moisture	5.94	g/100g
Crude fibre	23.10	g/100g

**Table 3:Effect of various treatments on betalain powder overall acceptability score.**

Treatment notion	Treatment combination	Beetroot powder overall acceptability score			
		Storage period (Days)			
		0 Days	30 days	45 days	60 days
<b>T<sub>0</sub></b>	Ground-Betalai(without treatment)	6.78	6.30	6.20	6.10
<b>T<sub>1</sub></b>	Ground - betalain + 1gm corn starch	7.40	7.30	7.21	7.11
<b>T<sub>2</sub></b>	G.-betalain + 2gm corn starch	7.30	7.10	7.02	6.93
<b>T<sub>3</sub></b>	G.-betalain + 3gm corn starch	8.54	8.24	8.10	8.01
<b>T<sub>4</sub></b>	Boiled – betalain + 1gm corn starch	7.16	6.80	6.72	6.61
<b>T<sub>5</sub></b>	Boiled – betalain+ 2gm corn starch	7.30	7.11	7.03	6.97
<b>T<sub>6</sub></b>	Boiled- betalain +3gm corn starch	8.45	8.15	8.04	7.91
<b>T<sub>7</sub></b>	Freezed- betalain +1gm corn starch	7.55	7.20	7.11	6.09
<b>T<sub>8</sub></b>	Freezed – betalain +2gm corn starch	7.90	7.70	7.63	7.51
<b>T<sub>9</sub></b>	Freezed – betalain +3gm corn starch	8.54	8.24	8.10	8.01
<b>Mean</b>		7.07	7.42	7.32	7.13
<b>C.V.</b>		2.67	2.99	2.10	2.99
<b>F' Test</b>		S	S	S	S
<b>S.E.(d)</b>		0.11	0.12	0.08	0.12
<b>C.D. at 5%</b>		0.35	0.37	0.26	0.36

#### **pH of betalain powder at different days of storage**

In freshly prepared betalain powder, mean pH value was observed to be 6.74, Significantly minimum pH was recorded in treatment T<sub>0</sub> Ground-Betalain (without organic corn starch) (6.16) followed by T<sub>6</sub> Boiled-Betalain + 3gm corn starch (6.67). However, significantly maximum pH was recorded T<sub>9</sub> freezed betalain + 3gm corn starch (6.94).After 30 days of storage, mean pH value was observed to be 6.44, ,maximum pH was recorded T<sub>9</sub> freezed betalain + 3gm corn starch (6.63).After 45 days of storage, mean pH value was observed to be 6.33, maximum pH was recorded T<sub>9</sub> freezed betalain + 3gm corn starch (6.50).After 60 days of storage, mean pH value was observed to be 6.12, minimum (5.34) pH was recorded for treatment T<sub>2</sub> G. betalain + 2gm corn starch followed by T<sub>0</sub> Ground-Betalain (without organic corn starch). However, significantly maximum pH was recorded T<sub>9</sub> Freezed betalain + 3gm corn starch (6.45).

**Table 5: Effect of different temperature and different parameters on Beetroot leather at different storage period.**

Parameters	Beetroot leather									
	At Room Temperature					At Cold Temperature				
	Storage period (Days)					Storage period (Days)				
	0 Days	30 days	45 days	60 days	Mean	0 days	30 days	45 days	60 days	Mean
<b>pH</b>	6.50	5.80	5.75	5.60	5.91	6.05	5.95	5.85	5.65	5.87
<b>T.S.S.</b>	72.12	72.35	72.71	73.24	72.60	72.12	72.25	72.74	73.24	72.58
<b>Ascorbic acid</b>	3.62	3.57	3.60	3.58	3.59	3.62	3.64	3.61	3.59	3.61
<b>Moisture</b>	14.80	14.67	14.32	14.15	14.48	14.80	14.74	14.51	14.27	14.58
<b>Total Sugar</b>	62.82	63.55	63.61	63.75	63.43	62.82	63.34	63.43	63.54	63.28
<b>Colour and Appearance score</b>	8.90	8.50	8.20	7.90	8.37	8.80	8.70	8.50	8.20	8.55
<b>Aroma score</b>	8.20	7.98	7.71	7.59	7.87	8.20	8.10	7.90	7.80	8.00
<b>Taste score</b>	8.80	8.50	8.10	7.90	8.32	8.80	8.60	8.40	8.20	8.50
<b>Texture score</b>	8.00	7.50	7.20	6.20	7.22	8.85	8.85	8.80	8.75	8.81
<b>Overall acceptability score</b>	8.60	8.20	8.10	7.90	8.20	8.90	8.60	8.40	8.20	8.52
<b>Mean</b>	19.92	19.76	19.62	19.48		19.93	19.96	19.90	19.84	
<b>C.V.</b>	4.51	2.91	6.11	6.01		3.75	4.76	2.51	2.96	
<b>F' Test</b>	S	S	S	S		S	S	S	S	
<b>S.E.(d)</b>	0.51	0.33	0.69	0.67		0.43	0.55	0.28	0.34	
<b>C.D. at 5%</b>	2.08	0.98	2.04	1.99		1.27	1.62	0.85	1.36	

### Conclusion

On the basis of experiment conducted, it is concluded that the best method to extract betalain was by freezing – ground and squeezing method. Treatment T<sub>9</sub> (Freezed-Betalain + 3gm corn starch) was found superior for preparation of beetroot powder in terms of physio-chemical properties, pH, TSS and organoleptic test viz. color, appearance, texture and taste. The maximum Benefit cost ratio (2.46) was also found on treatment T<sub>9</sub> (Freezed Betalain + 3gm corn starch).

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