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Different Methods of Betalain Extraction from Red Beetroot (*Beta vulgaris* L.) for Preparation of Solid Food Colour and Preparation of Beetroot Leather

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 (530m/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 3g corn starch (T^9) was found best for betalain powder prepration in terms of physio-chemical properties, pH and organoleptic test viz. color and appearance, texture and taste. The combination of beetroot pulp 860g + 1g salt + 1g citric acid + 200g sugar + 4g 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 T9 (Freezed Betalain + 3g corn starch).

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1. INTRODUCTION

Red beetroot, botanically known as *Beta vulgaris L*., belongs to the goosefoot (*Amaranthaceae*) family. Beetroot is a vegetable consumed worldwide due to its high content biologically active substances, such as betalain, folates, protein, iron, inorganic nitrates, as wellas minerals and vitamins present in the tuberous root [1,2].

Betalain is the major pigments present abundantly in beetroot which are chemical defined as the derivatives of betalanic acids [3]. Betalains impart a desirable red colour to food and have numerous applications in food industry as additives in gelatins, desserts, confectioneries, baked foods, etc [4]. Betalains have received an increased attention due to their antiviral and antimicrobial activities. The stability of betalains is strongly influenced by sugar, light, oxygen, water activity, pH and temperature [3]. All betalain pigments are water soluble, a property exploited to extract the pigment.

Betalains are immune derivatives of betalanic acid. They can be divided in two structural groups, the yellow betaxanthins and red-purple betacyanins [5,6]. Betalains accumulation in red beet root is related to the storage of carbohydrates, as a physiological response under stress conditions. Betalains have no toxic effects in the human body and represent a safe natural alternative to some synthetic colour additives, which are currently in use [7-10].

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) [11]. Beetroot has excellent physiological properties.

The intense red colour of beetroots derives from high concentrations of betalains, a group of phenolic secondary plant metabolites [12,13]. They are mostly cultivated for the commercial production of juices, concentrates and powders which are further used in culinary, confectionaries, manufacturing of sugars, pharmaceuticals, and dairy (Stinzing and Carle, 2007).

For quality evaluation of food, color is the primary screening for consumers. The application of colors to food includes uniformity of color across batches of a product, providing color to colorless foods and restoring the original color of a food [14]. Natural color in the form of pigments is synthesized and accumulated in living biological cells of algae, vertebrates, invertebrates, fungi, lichens, or bacteria. The red color in food industry comes mainly from two pigments: anthocyanin and betalain [15-18]. Betalain, derived from beetroot are water-soluble nitrogenous pigments that stop or delay the oxidation process and exhibit anti-tumor and anti-atherosclerotic effects [19,20]. The commercial beetroot red (mainly betalain) is permitted widely in dairy products such as ice cream, sherbet, and yogurt, dry soft drink mix along with confectionaries, soups, and bacon products. Betalain are relatively pH stable (range of 3–7) [21] and contain a multitude of health benefits including their antioxidant, anticancer and anti-diabetic activity [22]. Betalain are mainly composed of red-violet betacyanin and yellow-orange betaxanthin containing betalamic acid as the bioactive unit. The stability of betalain varies with different levels of water activity, temperatures, exposure to oxygen, and light. The richest natural sources of betalain include red beet, Amaranthus, *Hylocereus polyrhizus* (red pitaya), and *Opuntia ficus* (prickly pear).

Natural colourants have gained popularity in recent years, owing to their purported lack of toxicity and eco-friendliness [23-25]. Natural colours have been discovered to be nutritional antioxidants, and their inclusion in the diet can lower the risk of cardiovascular disease, cancer, and disorders connected with ageing [3]. When the red beet was first introduced to Britain in the 17th century, it was characterised as the most excellent and delicate salad. With intriguing new nutrition research backing its significant health advantages, this humble root vegetable is making its way out of conventional recipe books and onto the menus of the city's hippest eateries [26-28].

I have selected the work on the extraction of Betalain & preparation of betalain powder and beetroot leather from Beta Vulgaris because to till date this work has not been done by any researcher [29,30]. Therefore, the purpose of this work is to use Beta Vulgaris extract as a cheap source of solid food colour which has no harmful effect for health.

2. MATERIALS AND METHODS

2.1 Experimental Site

An experiment on "Different extraction methods of Betalain from red beetroot (*Beta vulgaris* L.)for preparation of solid food colourant and preparation of Beetroot Leather" was laid out with the appropriate methodology at the post-harvest laboratory of Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh during 2021-2022.

2.2 Materials Used

All materials used in extraction of Betalain and preparation of betalain powder and beetroot leather from red beetroot were purchased from the local market and local farmer's field of prayagraj district. The instruments used in extraction of betalain and preparation of betalain powder and leather such as juicer, refrigerator, induction, weighing machine, steel containers, PH meter, Refractometer etc.

Table 1. Materials used in extraction of betalain and preparation of betalain powder and beetroot leather from beetroot

Material used	Quantity
Beetroot	5 kg
Organic cornstarch	20 g
Matodextrin powder	4 g
Citric acid	1 g
Salt	1g
sugar	200g

2.3 Experimental Procedure of Betalain Extraction

Betalain extracts were prepared from 1 kg of fresh beetroots for each method. Three methods are used for the betalain extraction. Beetroots were peeled, ground, squeezed and betalain was collected in beaker. In the 1st step ground 850g/1kg (after peeling) beetroots and extracted betalain by squeezing through muslin cloth manually, 510m betalain was collected in a beaker, in 2nd step beetroots 1kg beetroot peeled 820g 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 290m betalain collected, in 3rd step 845g beetroots were cut in to small pieces and freezed at -20ºC in a commercial freezer for 12-14 hr, 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 530m with dark colour.

2.4 Methodology for Preparation of Betalain Powder

After extraction of betalain by following above 3 steps extracted betalains were devided 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.

2.5 Methodology: Flowchart of Preparation of Beetroot Leather

2.5.1 pH

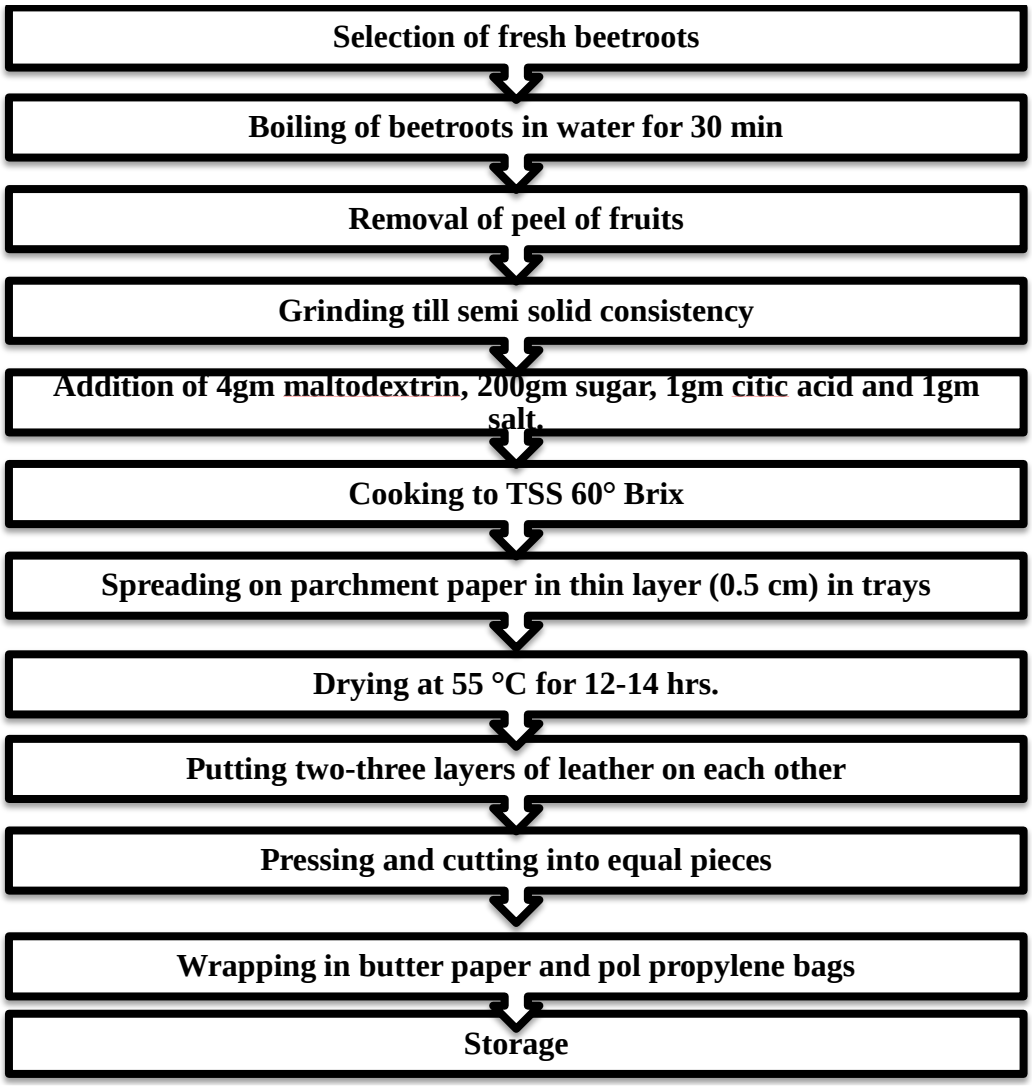
The pH was determined using a pH meter; the pH meter was standardized using standard buffer of pH 4. The sample was taken in a beaker, the electrodes of the pH meter were dipped into it for 1 minute and the pH was recorded. The electrodes of the pH meter were washed with distilled water after each determination.

2.5.2 Total soluble solid (TSS)

The total soluble solids in beetroot leathers were determined by weighing the ground sample and mixing in 20 ml distilled water and kept for 1 hour. After standing it was centrifuged at 5000 rpm for 5 min (by using research centrifuge R-24). The supernatant was taken in petri dish and evaporated to solid form with constant weight. Weight of total soluble solids was recorded in grams and expressed as gram per 100 g sample.

2.5.3 Moisture

The initial moisture content of leather was determined by standard oven method [31. 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.



Flowchart 1. Preparation of beetroot leather

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

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.

2.5.4 Betalain

The total betalain pigment was determined by the methods reported by Stintzing et al. [32].

2.5.5 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).

2.6 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 analyzed with the help of 'F' (Variance ratio) test the significant differences between the means were tested with the critical differences at 5% probability level.

2.6.1 Storage of betalain powder and beetroot leather

Beetroot leather samples were stored as per treatment at ambient (28 ± 2 °C) and refrigerator temperature (5 ± 2ºC) for 60 days. The samples were drawn at an interval of 30 days, 45 days and 60 days for chemical analyses, sensory evaluation and microbial quality.

2.6.2 Sensory evaluation

Sensory evaluation of beetroot leather was carried out according to method of Amerine et al. [33] on a 9-point hedonic scale. The average scores of the nine semi-trained judges for different quality characteristics viz. colour and appearance, flavor, taste, texture and overall acceptability were recorded.

2.6.3 Packaging of betalain powder and beetroot leather

Polypropylene pouches and air tight containers with siliqa gel are used to store.siliqua gel helped to keep the samples free from moisture.

3. RESULTS AND DISCUSSIONS

In this study, from the T_9 freezed betalain method we collected highest concentrated betanin formulation, betalain content was 13.62mg/100g. Different experimental conditions was performed by extracted betalain to prepare betalain powder by using different concentration of organic cornstarch and we got excellent amount of solid food colour with good source of nutritional value also from the beetroot leather. The nutritional values of betalain powder and beetroot leather are presented in Table 2.

In freshly prepared betalain powder, mean pH value was observed to be 6.74, Significantly minimum pH was recorded in treatment T0 Ground-Betalain (without organic corn starch) (6.16) followed by T6 Boiled-Betalain + 3gm corn starch (6.67). However, significantly maximum pH was recorded T9 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 T9 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 T9 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 T2 G. betalain + 2gm corn starch followed by T0 Ground-Betalain (without organic corn starch). However, significantly maximum pH was recorded T9 Freezed betalain + 3gm corn starch (6.45).

Table 2. Effect of Nutritional compositions of betalain solid food colour and beetroot leather yield by different methods of extracted betalain and beetroot pulp (g/100gm)

Sl no	Test parameters	Test Methods	Results	Unit
		Beetroot solid		
01.	Energy	FSSAI Lab Manual Method	350.82	Kcal/100gm
02.	Carbohydrate	FSSAI Lab Manual Method	72.71	g/100g
03.	Protein	Kjeldhal method	12.52	g/100g
04.	Fat	Soxhlet method	1.10	g/100g
05.	Ash	FSSAI Lab Manual Method	7.73	g/100g
06.	Moisture	FSSAI Lab Manual Method	5.94	g/100g
07.	Crude fibre	FSSAI Lab Manual Method	23.10	g/100g
		Beetroot Leather		
01.	Energy	FSSAI Lab Manual Method	338.83	Kcal/100gm
02.	Carbohydrate	FSSAI Lab Manual Method	75.94	g/100g
03.	Protein	Kjeldhal method	18.52	g/100g
04.	Fat	Soxhlet method	0.11	g/100g
05.	Ash	FSSAI Lab Manual Method	2.34	g/100g
06.	Moisture	FSSAI Lab Manual Method	13.09	g/100g
07.	Crude fibre	FSSAI Lab Manual Method	1.59	g/100g

**NOTE – The samples has been analyzed from 'FOOD ANALYSISAND RESEARCH LABORATORY, Centre Of Food Technology, Institute of professional studies, science faculty campus, University of Allahbad'*

Table 3. Effect of various treatment combinations on pH of betalain powder at different days of storage

Treatments	Treatment combinations	pH			
		Storage period (Days)			
		0Days	30days	45days	60days
T0	GroundBetalain(withouttreatment)	6.16	6.05	5.90	5.80
T1	Ground-betalain + 1g corn starch	6.80	6.51	6.42	6.33
T2	Ground-betalain + 2g corn starch	6.89	6.57	6.43	5.34
T3	Ground-betalain + 3g corn starch	6.88	6.47	6.31	6.17
T4	Boiled – betalain + 1g Corn starch	6.77	6.44	6.33	6.20
T5	Boiled – betalain+ 2g corn starch	6.70	6.40	6.30	6.18
T6	Boiled- betalain +3g corn starch	6.67	6.38	6.25	6.15
T7	Freezed- betalain +1g corn starch	6.81	6.51	6.44	6.32
T8	Freezed – betalain +2g corn starch	6.85	6.50	6.42	6.29
T9	Freezed – betalain +3g corn starch	6.94	6.63	6.50	6.45
Mean		6.74	6.44	6.33	6.12
C.V.		3.22	2.62	1.78	2.60
F' Test		S	S	S	S
S.E.(d)		0.12	0.09	0.06	0.092
C.D. at 5%		0.37	0.28	0.19	0.27

Table 4. Effect of various treatment combinations on colour and appearance score of betalain powder at different days of storage

Treatments	Treatment combinations	Colour and appearnce score			
		Storage period (Days)			
		0Days	30days	45days	60days
T0	Ground-Betalain (without treatment)	6.99	6.79	6.67	6.40
T1	Ground - betalain + 1g corn starch	7.10	7.05	6.10	6.05
T2	G-.betalain + 2g corn starch	7.55	7.50	7.43	7.30
T3	G.-betalain + 3g corn starch	8.49	8.30	8.23	8.29
T4	Boiled – betalain + 1g corn starch	7.40	7.30	7.21	7.11
T5	Boiled – betalain+ 2g corn starch	7.66	7.56	7.50	7.45
T6	Boiled- betalain +3g corn starch	7.77	7.60	7.56	7.40
T7	Freezed- betalain +1g corn starch	7.60	7.56	7.42	7.30
T8	Freezed – betalain +2g corn starch	7.80	7.70	7.59	7.41
T9	Freezed – betalain +3g corn starch	8.65	8.55	8.45	8.30
Mean		7.70	7.59	7.41	7.30
C.V.		2.06	1.90	2.65	3.07
F' Test		S	S	S	S
S.E.(d)		0.09	0.08	0.11	0.13
C.D. at 5%		2.71	2.46	0.33	0.38

In freshly prepared betalain powder i.e. at 0 days, mean Texture score value was observed to be 7.76, C.V. was recorded with value of 2.87 while S.E.(d) was recorded to be 0.12. Significantly minimum Texture score was recorded in treatment T0 Ground-Betalain (without organic corn starch) (6.86) followed by T4 Boiled-Betalain + 1gm corn starch (7.17). However, significantly maximum Texture score was recorded T9 freezed betalain + 3gm corn starch (8.74). Significantly after 30 days of storage, maximum Texture score was recorded T freezed betalain + 3gm corn starch (8.18). significantly after 45 days of storage significantly maximum Texture score was recorded T9 freezed betalain + 3gm corn starch (8.18).significantly after 60 days of storage, minimum Texture score was recorded in treatment T1 Ground-Betalain + 1gm corn starch (6.34) followed by T0 Ground-Betalain (without organic corn starch) (6.34). However, significantly maximum Texture score was recorded T9 freezed betalain + 3gm corn starch (8.02).

Table 5. Effect of various treatments on betalain powder overall acceptability score by physical appearance

Treatment notion	Treatment combination	Beetroot powder overall acceptability score			
		Storage period (Days)			
		0 Days	30 days	45 days	60 days
T0	Ground-Betalai(without treatment)	6.78	6.30	6.20	6.10
T1	Ground - betalain + 1g corn starch	7.40	7.30	7.21	7.11
T2	G-.betalain + 2g corn starch	7.30	7.10	7.02	6.93
T3	G.-betalain + 3g corn starch	8.54	8.24	8.10	8.01
T4	Boiled – betalain + 1g corn starch	7.16	6.80	6.72	6.61
T5	Boiled – betalain+ 2g corn starch	7.30	7.11	7.03	6.97
T6	Boiled- betalain +3g corn starch	8.45	8.15	8.04	7.91
T7	Freezed- betalain +1g corn starch	7.55	7.20	7.11	6.09
T8	Freezed – betalain +2g corn starch	7.90	7.70	7.63	7.51
T9	Freezed – betalain +3g corn starch	8.54	8.24	8.10	8.01
Mean		7.07	7.42	7.32	7.13
C.V.		2.67	2.99	2.10	2.99
F’ Test		S	S	S	S
S.E.(d)		0.11	0.12	0.08	0.12
C.D. at 5%		0.35	0.37	0.26	0.36

Table 6. Effect of different temperature and different parameters on (T_{10}) 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
pH	6.50	5.80	5.75	5.60	5.91	6.05	5.95	5.85	5.65	5.87
T.S.S.	72.12	72.35	72.71	73.24	72.60	72.12	72.25	72.74	73.24	72.58
Ascorbic acid	3.62	3.57	3.60	3.58	3.59	3.62	3.64	3.61	3.59	3.61
Moisture	14.80	14.67	14.32	14.15	14.48	14.80	14.74	14.51	14.27	14.58
Total Sugar	62.82	63.55	63.61	63.75	63.43	62.82	63.34	63.43	63.54	63.28
Colour and Appearance score	8.90	8.50	8.20	7.90	8.37	8.80	8.70	8.50	8.20	8.55
Aroma score	8.20	7.98	7.71	7.59	7.87	8.20	8.10	7.90	7.80	8.00
Taste score	8.80	8.50	8.10	7.90	8.32	8.80	8.60	8.40	8.20	8.50
Texture score	8.00	7.50	7.20	6.20	7.22	8.85	8.85	8.80	8.75	8.81
Overall acceptability score	8.60	8.20	8.10	7.90	8.20	8.90	8.60	8.40	8.20	8.52
Mean	19.92	19.76	19.62	19.48		19.93	19.96	19.90	19.84	
C.V.	4.51	2.91	6.11	6.01		3.75	4.76	2.51	2.96	
F' Test	S	S	S	S		S	S	S	S	
S.E.(d)	0.51	0.33	0.69	0.67		0.43	0.55	0.28	0.34	
C.D. at 5%	2.08	0.98	2.04	1.99		1.27	1.62	0.85	1.36	

Beetroot powder can be stored upto 60 days. Colour change was observed during that period from red brown to dark brown.

In beetroot leather prepared i.e. T10 (Beetroot pulp 860gm + 1gm salt + 1gm citric acid + 200gm sugar + 4gm maltodextrin powder) at Cold temperature, pH gradually decreased from 6.05 recorded at 0 days to 5.65 recorded at 60 days after storage. The T.S.S. value increased from 72.12 recorded at initial day i.e. 0 day to 73.24 recorded at 60days after storage. Similarly Ascorbic acid content of beetroot leather also decreased from 3.62 value at 0 day to 3.59 value recorded at 60 days after storage. The moisture content of the beetroot leather decreased from 14.80% at 0 day to 14.27% at 60 days after storage. Total sugar was observed to be decreasing first and then increasing from day 0 (recorded 62.82) followed by day 30 (recorded 63.34) and then again increased at day 60 (recorded 63.54). Colour and appearance score of beetroot leather decreased from day 0 recorded value 8.80 to 8.20 value at 60 days after storage. Aroma score also decreased from day 0 (recorded value 8.20) to 60 days after storage (recorded value 7.80). Taste score decreased from its value of 8.80 on day 0 to 8.20 value on 60 days after storage. Score for texture was observed to be 8.85 on initial day and it decreased with record of 8.75 at 60 days after storage. Overall acceptability score of beetroot leather also decreased from 8.90 value on 0 day to 8.20 value on 60 days after storage.

Similar results were reported for total sugar of fig leather by Kotlawar et al. (2011); total sugar of jackfruit leather by Khadatar [34]; total sugar of guava leather by Chavan and Shaik [35]; total sugar of wood apple Totre (2016).

4. CONCLUSION

On the basis of experiment conducted, it is concluded that the best method to extract betalain was by freezing – ground and squeezing method, higher concentration of betalain extracted due to freezing of beetroots. Treatment T9 (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 T9 (Freezed Betalain + 3gm corn starch), Also T_{10} (Beetroot leather) was found superior by taste and appearance maltodextrin showed better organoleptic properties as well as chemical composition and good storage stability at both storage (ambient and refrigerated) conditions up to 3 months of storage period.

Further research on the manufacturing of betalain powder and leather on a pilot scale, as well as consumer acceptability studies, are required for good storage durability, better beetroot utilisation for enhancing people's nutrition, and this will offer new paths for commercial beetroot utilisation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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