DEVELOPMENT OF NATURAL JELLY FROM BEETROOT PEEL: ANALYZE ITS STABILITY AND SENSORY PROPERTIES

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ABSTRACT

The present research work was made to formulate and evaluate natural jelly from Beetroot peel. Beet root (*Beta vulgaris*) is a vegetable with high amount of biologically active substances and inorganic nitrogen. Ultra sonication assisted aqueous extraction of pigment from beetroot peel was done and total betalain pigments were measured by pH differential method. FTIR results confirm the presence of amine and carboxyl group which indicates the medicinal and nutritive value of beetroot peel. In this study two different formulations (FI and FII) were prepared by using agar agar (Food grade), brown sugar, honey, citric acid and salt. Stability of the betalain pigment was analyzed at various temperature (5, 10, 15, 20, 25 and 30°C) also evaluated antimicrobial and antioxidant property. The results of the storage properties revealed that the developed jelly can be stored under refrigerated and ambient conditions for a period of 15days respectively without addition of any artificial preservative. Based on the sensory quality characteristics viz., color, texture, shape, flavor and overall acceptability FI can be used in development of jelly successfully.

Keywords: Beetroot peel, Jelly, Betalain, Antimicrobial, Antioxidant, Sensory analysis

INTRODUCTION

Beetroot (*B. vulgaris*) is biennial plant grown in temperate countries and it is an alkaline food with pH in range of 7.5 to 8.0 [33]. Beet root are the excellent source of beta carotene, calcium ion and nitrogen. In India it is mainly grown for its juice and vegetable value which contains vitamin A, B1, B2, B6 and C also it is the good source of various minerals [24]. Recent report indicates that beet root extract possesses anti-hypertensive, hypoglycemic, anti-oxidant, anti-inflammatory, and hepatic protective activity additionally beetroot is the natural source of dye and it is used for many food processing unit. The pigment present in the beet root is betalain, an alkaline pigment and is a glucoside that hydrolyzes into sugar glucose and betanidin [42]. Generally, betalain has excellent stability at various pH, light and temperature.

Most of the beet products are made from sugar beet and most importantly beet cellulose works as a bulk residue, increasing peristalsis and making stool passage easier, hence constipation is prevented by using it on a regular basis also, lowers blood pressure in hypertensive persons. Gastric ulcer can be treated with beetroot wine. It increases the urinary output due to its rich potassium content and cures hypo-glycaemia. It is also helpful in various treatment of piles, tuberculosis, cholera, diarrhea, dysentery and lowered state of body resistant after major surgical operation etc [41]. The beet root juice is a natural cure for erectile dysfunction and the removal of kidney and bladder stones [37].

Waste material of beet root peel high concentration of phenolic compounds, it is a rich source of phytochemicals and antioxidants. [15]. Vulgaxanthin I, Vulgaxanthin II, indicaxanthin, betanin, prebetanin, isobetanin, and neobetanin were among the betalains discovered in beetroot peel extract. cyclodopa glucoside, V-formylcyclodopa glucoside, dihydroxyindolcarboxylic acid glucoside, betalamic acid, L-tryptophan, p-coumaric acid, ferulic acid, and quantities of unidentified flavonoids are also present. [21]. In the recent report, green synthesis of nanoplates from aqueous extract of beet root peel was investigated for anisotropic noble metal research (Deokar and Ingale, 2018). Due to higher antioxidant level and nitrogen is used as a good quality fertilizer than that of standard antioxidants [3].

Tomato pastes, soups, sauces, desserts, jams, jellies, candies, and morning cereals all benefit from beet root peel extract. Jellies are made by cooking fruit juice with sugar. It is made from juice or aqueous extract of one or more fruits or vegetables with sweetening properties with or without

the addition of water [13]. Kids of all ages like jelly but commercial jellies containing high amounts of artificial colorants, sugar or high fructose corn syrup can be detrimental to one's health.

The purpose of this research was to develop and assess natural jelly from beet root peel with the help of sweeting agent and gelling agent and thereby sensory analysis carried out.

MATERIALS AND METHODS

Collection and Storage of Samples

Beetroot (Vitamin A, C and B6), Agar agar (Gelling agent), citric acid or lemon, brown sugar and Honey (Sweetening agent) procured from Reliance fresh Mart, Coimbatore, Tamilnadu, India. The peel was removed and dried under hot air oven at 75ºC for 2 days. The dried peel was finely powdered in a grinder and stored in a container for further studies [12].

Pigment Extraction

In a conical flask, ten grams of powder were concentrated with 50 ml of deionized water. The mixture was then processed under sonicator for about 6 hrs under proper conditions and observed in proper intervals of time. The aqueous extract was then centrifuged for 10 minutes at 5000 rpm and the supernatant is collected and stored in refrigerator [1].

Phytochemical analysis

Phytochemical analysis of the beetroot peel extract was carried out in accordance with standard Protocol [18].

Total pigment content Estimation

Total amount of Betalain pigment was measured by the [8] method with modified dilution.

$$\textbf{Total pigment content } \left(\frac{mg}{g}\right) = \frac{A \times DF \times MW \times 1000}{\varepsilon \times L}$$

Chemical Structure Analysis

FTIR (Fourier Transform Infrared Spectroscopy)

The extracted pigments' Fourier transform-infrared spectrum was obtained from pellets made with 1 mg of sample and 100 mg of dry potassium bromide (KBr). The spectra were taken with a Perkin Elmer FT-IR Spectrum GX (USA) infrared spectrophotometer between 700 and 4000 cm^{-1}. [9].

Antimicrobial Activity

The agar well diffusion method was used to test the antimicrobial activity of the peel extract (1.0 mg). The activity was evaluated against two Gram- Negative bacteria (*Escherichia coli* MTCC 1687, *Serratia marcescens* MTCC 4822), two Gram-positive bacteria (*Bacillus cereus* MTCC 6840 and *Staphylococcus* aureus MTCC 96), two microscopic filamentous fungi (*Aspergillus flavus* MTCC 535 *and A. versicolor* MTCC 698). The Bacteria and fungi were collected from Microbial Type Culture Collection, Chandigarh**.** Microbial growth inhibition was measured around the impregnated discs. When the zone diameter is > 10 mm, 5–10 mm, or 2–5 mm, antimicrobial activity is termed high, moderate, or trace, respectively, and minimal effect when the value is less than 2 mm and % of inhibition was computed. [9].

Anti-oxidant Property

DPPH Radical Scavenging Method

DPPH scavenging activity of Betalain pigment was tested by the method of Sanchéz-Moréno *et al*., (1998). For anti-oxidant study, dried extract was used and the method was described in [19]. DPPH solution in methanol (0.1mM) was mixed with different concentration of extract (20 to 100mg/l) and read the absorbance at 518 nm. The standard utilized in this investigation was 1, 4-dithioerythritol, and the results were given in milligrams per liter. [16].

Thin Layer Chromatography

Separation of Betalain pigment was done by cellulose coated TLC plate with the sequential use of two different polar system (Less PS I - Ethanol: Acetic acid: Isopropanol: water: 20:5:55:20 ml) and More PS II - Isopropanol: Ethanol: Water: HCl: 55: 20: 24: 1 ml). A hundred milliliters of beetroot extract were combined with two milliliters of water. and applied on the TLC plate and allowed the solvents moved 10cm then dried under atmosphere and run twice in two different solvents in the same direction. This method was done with modified procedure of [6].

Preparation of Formulation for organoleptic evaluation

Two different formulations were prepared with various concentrations of the ingredients for organoleptic evaluation studies (Tab 1).

Table 1. Formulation based various combination of the extract

S.No	Sample	FI	FII
1.	Beet root extract (ml)	5	10
2.	Agar agar (g)	2	2
3.	Honey (ml)	10	5
4.	Brown Sugar	10	5
5.	Water (ml)	3	3
	Total	20	20

Preparation of Jelly

Based on organoleptic evaluation the selected formulation was heated and filled in small cups (preferably used for jelly preparation) and stored in refrigerator for 1 hr. The prepared jellies can be scooped from the cups and can be consumed directly [10].

Stability and Storage Study

The selected formulation was checked for stability at various temperatures (5, 10, 15, 20, 25 and 30˚C) samples were placed in capped glass vials wrapped with aluminium foil and sealed with parafilm during the storage test for 30 days by measuring the samples in colorimeter at 535nm. The reading was taken at the interval of 5 days once until 30 days.

Statistical Analysis

For significant differences at $p<0.05$, the data were subjected to one-way ANOVA followed by post hoc Duncan's Multiple Range Test (DMRT) using SPSS 17.

RESULTS AND DISCUSSION

Sample Extraction & Preliminary Screening

The beetroot peel was finely powdered and extracted with d.H_2O under ultrasonicator for 6h, centrifuged and stored the supernatant under refrigeration (Plate 1). The Extract was subjected for different chemical analysis for preliminary screening and the results indicated the presence of various compounds and shown in table 2. It has been reported extensively, that betalain pigment

was extracted from beetroot peel by ultra-sonication method with the extraction time of 3h and stored at low temperature [38]. Similarly, ultrasonication assisted distilled water extraction was carried out and reported as this method was not significantly influence on pH, soluble solids and total solids but yield of pigment was significantly higher [27]. According to the method of [39] ultrasound-assisted extraction showed better results in betalain extraction than orbital shaking extraction. Our report is extensively correlated with the results of [18, 30] for the presence of flavonoids, saponins, sterols and triterpenes in beetroot extracts after phyto-chemical screening. This type of biochemical profile has been observed in another study using GC-MS that dealt with *Satureja khuzistanica* for preserving vegetable oils [4]. Phytochemicals of different beetroot's vegetation extract were analyzed and reported [5].

Plate 1: Ultrasonicated Aqueous Extract

Phytoconstituents	Qualitative Analysis
Tannins	+
Saponins	+
Flavonoid	+
Reducing sugars	+
Terpenoids	+
Triterpenes	+
Anthraquinones	-

Table 2: Phytochemical analysis of the extract

Chemical structural analysis

The FTIR images showed two strong bands at 3309.39 cm^{-1}, and 1635.40 cm^{-1}corresponding to N-H stretch for 1º, 2ºamines and C=O stretch for carboxyl group respectively were observed. (Figure 1).

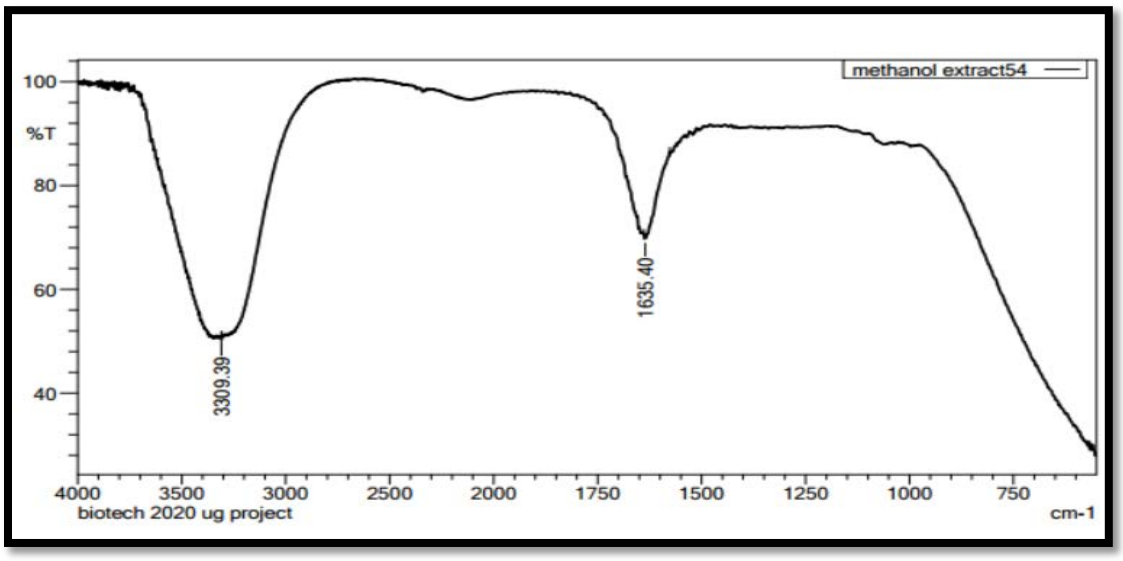


Figure 1. FTIR analysis

From figure 1, amines & carboxyl groups present in the extract possessed the use of medicines, food product preparation and source of good preservatives respectively. Also, the incidence of nitrogen functional groups confirmed the existence of betalains which is similar to the findings of [11] at 1,415 $cm^{-1.}$ The transmittance results at 3319 and 1637 cm1 were consistent with our findings, indicating asymmetric and symmetric stretching vibrations of OH groups and H-O-H due to moisture content [26]. In beet juice lyophilization study, show the stretching of -OH group and NH at 3300 and 1620 cm^{-1}. The same method was used to detect the vibration frequencies of betalain functional groups, which revealed that the band at 3359 cm-1 was caused by the stretching vibration of the N-H bond. [22].

Anti-microbial activity

The antimicrobial effect of is shown in table 3. *E. coli* exhibited the maximum antimicrobial activity with inhibition of about 90.2±0.2 compared with the standard disc of Chloramphenicol (100%). *S. marcescens, B. cereus, A. flavus* and *A. versicolor* of fungi showed clear zone formation

of growth inhibition, the percentage was calculated with standard fluconazole. The extract showed trace amount of inhibition on *S. aureus* compared to other strains. The antimicrobial activity of one of the major ingredients for jelly preparation was quantified in terms of their ability to restrict the growth of the test strains. Beetroots' antibacterial action has been linked to the presence of phenolic chemicals, which prevent the growth of undesirable microbes. There have been a few researches on the antibacterial effectiveness of beetroot. [13] suggested that beetroot extracts showed excellent activity against tested microbes *E. coli, P. vulgaris, S. aureus, K. pneumonia and P. mirabilis*. The extract of 100 µl demonstrated inhibitory efficacy against all Gram–negative bacteria tested, with *Salmonella typhimurium* and *Citrobacter freundii* being the most vulnerable strains, with 50 µl of the extract inhibiting their growth [42]. Endometabolites of sugarbeet seed residues, such as phenols, flavonoids, and their glycosides, have antibacterial properties in some circumstances and can be exploited as natural biologically active chemicals. [28].

Microorganism	**Inhibition Zone (mm)**	**Inhibition (%)**
Bacteria		
Escherichia coli	H	90.2±0.2
Serratia marcescens	M	52.3±0.4
Bacillus cereus	M	58.3±0.3
Staphylococcus aureus	T	35.4±0.2
Fungi		
Aspergillus flavus	M	49.1±0.2
A. versicolor	M	47.2±0.4

Values are mean±standard of the three replicates

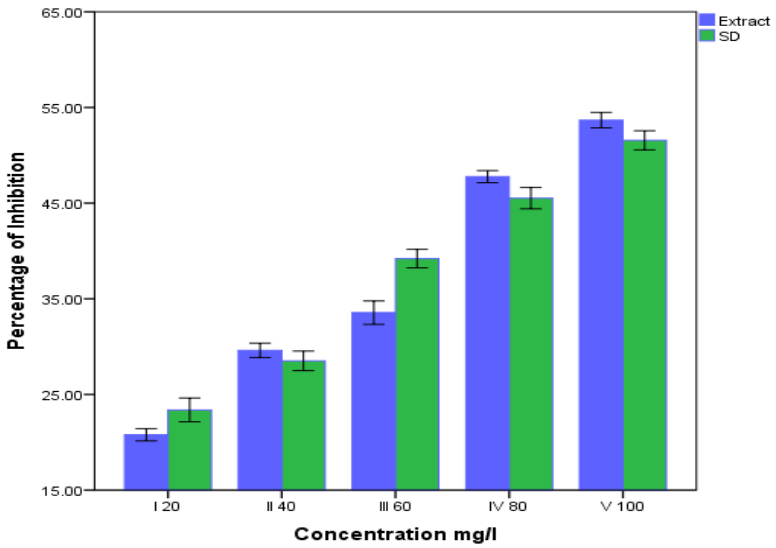
Legend: H > than 10 mm, M > 5–10 mm, T > 2–5 mm.

Table 3 Antimicrobial effect of Beetroot peel extract

DPPH Radical Scavenging Method

Aqueous extract of beetroot peel exhibited potent free radical scavenging activity by increasing the concentration from 20 to 100mg/l. It showed similar potential as standard 1, 4-dithioerythritol (20 to 100mg/l). IC_{50} value of the beetroot peel extract was 80 mg/l and for standard 91 mg/l

concentration. DPPH is dark purple color changes into yellow color by accepting hydrogen from donor and becomes a non-radical form (Figure 2). Similar to our study [34] reported, Methanol and ethanol beetroot extract displayed increasing DPPH scavenging activity when the concentration was increased and its IC50 values were 0.129 and 0.254 mg/ml. According to [23], increasing the concentration of beetroot peel extract boosted scavenging activity (4 to 20%).

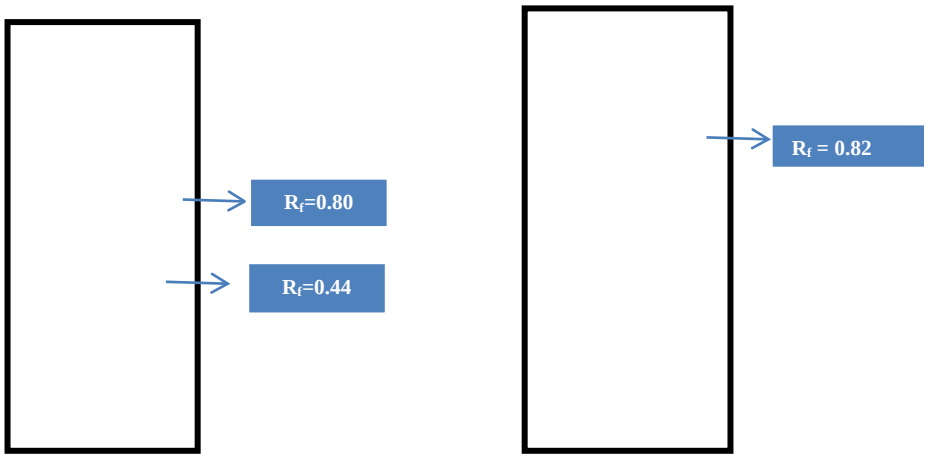


Legend: SD-Standard

Figure 2: DPPH scavenging property of Extract

Thin Layer Chromatography

TLC was used to validate the presence of betalain throughout the separation process. The separated pigment bands seen on a TLC plate by running with PS I and their corresponding visible spectra were identified as betacyanin pigment with identified red spot chromatogram by calculating the R_f value 0.80 and pink spot chromatogram identified as anthocyanin, R_f value found as 0.44. For PS II pink spot was identified with R_f value 0.82. By Interpreting the result with [18] the compound was confirmed to be betacyanin, R_f value was 0.98. Similar work was conducted on dragon fruit, different fraction has been shown, two have not been identified due to unavailable information. Known colored pigments betacyanin and anthocyanin was obtained with R_f value of 0.45 and 0.85 [31].



Legend: PS - Polar Systems

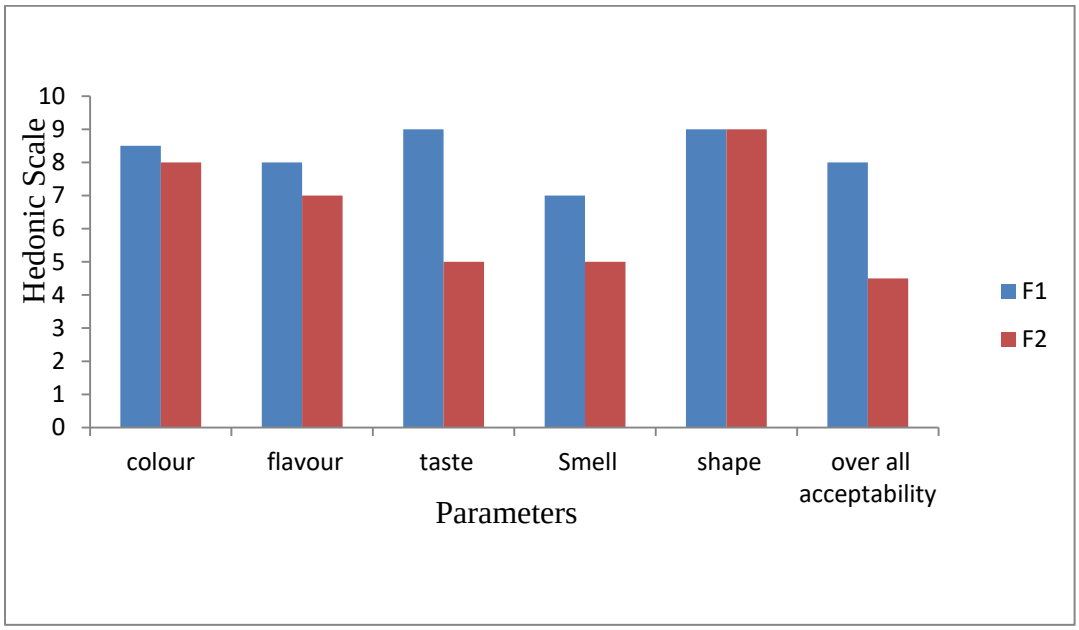
Plate 2: TLC of aqueous extract of beet root peel extract

Preparation of Jelly and Sensory Analysis

Two different formulations of jelly were prepared mentioned above in the method section. Mixed the ingredients and heated for 100°C 4 min or desired consistency was reached. Jelly was prepared and filled in small cup PS I y used for jelly preparation) a PS II n refrigerator for 1 hr and illustrated in figure 3. Previously, jelly preparation was done with beet root juice, pectin, citric acid and sugar and the highest evaluation score was 8.3 [10]. According to [38] natural jelly was prepared from citrus waste and analyzed carotenoid content. In another study, natural jellies were prepared with carrot, papaya and beet root with different ratios and its nutritive values were analyzed [17]. Different Sensory attributes like appearance, color, flavor, shape, texture and overall acceptability was analyzed by panel of 5 trained members having experience in sensory evaluations of the product with 9-point hedonic scale. Based on the average score formulation I was selected for further studies (Figure 4).

F1 **F2**

Figure 3 Jelly Preparation



Legend: F1- Formulation I; F2- Formulation II

Figure 4: Sensory Analysis of the formulations

Stability and Storage Test

Stability test of betalain for formulation I was performed at various temperatures and read the absorbance at 535nm for every 5th day until 30 days. Based on the report, the pigment was found to be stable up to 15ºC for 15 days and the stability decreases when the temperature and days increases above 15ºC and 15th day respectively. The hypochromic shifts occurs when the other components of

the sample react to the temperatures. The absorbance of samples stored at room temperature decreased gradually as the day increased but the jellies stored in refrigerator showed degradation of pigment after 15th day. Hence natural extract can be recommended to be used at or below 25ºC with minimum loss in pigments for 20 days (Figure 5). Previously the stability was tested with sugar free grape jelly for 60days at 4°C for diabetic patients or for individuals who desire to reduce their body weight [20]. The impacts of storage temperature and time of jam and juice from two varieties of monkey kola stored at different temperatures 29 to 32°C, there were no significant changes in specific gravity of the jam and juice samples stored for four weeks ($p>0.05$) [29].

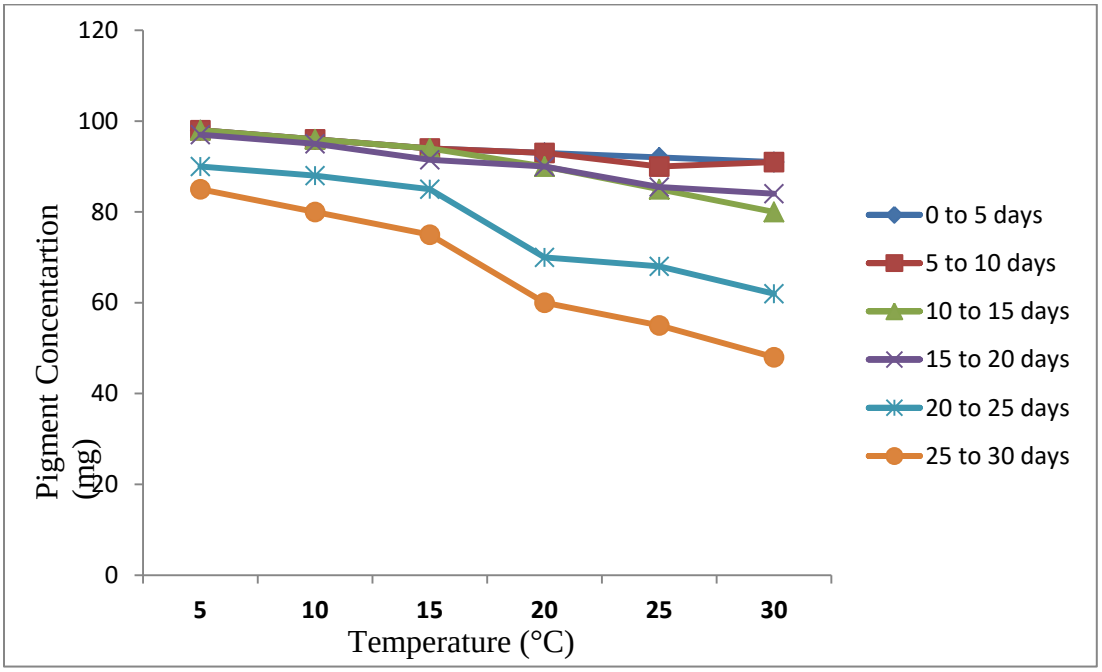


Figure 5: Stability study of the sample at various temperatures

PATENT DETAILS

B Vishnupriya, V Agalya "Fiber to Sugar free Jelly from By - Product of Beetroot", "202141025316", June 7, 2021.

Available: https://ipindiaservices.gov.in/PublicSearch/PublicationSearch/PatentDetails, Accessed on: June 11, 2021.

CONCLUSION

The focus of this research was on making jelly from beetroot peel. Because of the high concentration of phenolic compounds, the by-product of beet root peel contains rich source of phytochemicals and antioxidants. For the successful preparation of jelly, the formulation I was standardized as beetroot extract (5 ml), agar agar (2 g), honey (10 ml) and brown sugar (10 ml). The stability was checked at different temperature. The jellies stored in refrigerator and ambient condition showed pigment stability until 15^{th} and 5^{th} day respectively without addition of any artificial preservative. In sensory examination, the beetroot jelly formulation received the maximum score of 8.0 overall acceptance. The nutritional properties of the formulation will be analyzed in future.

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