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Study on the factors involved in Colour Stability of Betanin, a natural red colour from *Beta vulgaris* L.

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ABSTRACT
Natural colorants usages are increasing globally, leaving behind synthetic colourants in their usage, due to the realization that, they are safer, and ecofriendly, in nature. Among those natural colors, red dye is widely used and predominantly, it is from betanin derived from betalains, a natural dye pigment from *Beta vulgaris* L. (Amaranthaceae). The betanin are quite sensitive toward heat, pH, light, and oxygen, which lack in their stability. The current study clearly evolves the ideal temperature, pH to be maintained, colour index and the storage conditions, which are very much necessary, for developing a formulation, in cosmetics or food, using natural dye like betanin.
KEYWORDS: Natural red colour, Betalains, Betanin, Beet root, Natural pigments, *Beta vulgaris*.

I. INTRODUCTION:

Beetroot colour is widely used industrially, due to its red colour, from the pigment betalain mainly from betanin, betanidin, and betaxanthin [1].The usage of such color cosmetics has began since prehistoric times [2].The use of natural colorants in India, were observed since, Indus Valley Civilization [3]. Even some of the natural pigments used as colorants, like Henna and Saffron, were used as colorants around 2500 BC [4].

The pigments/colors used in cosmetics are mostly synthetic colors or dyes. Some of the widely used synthetic dyes in cosmetics are azo dyes, xanthone, quinoline, Indigoid, etc., [5]. Among these various synthetic dyes, azo dyes are found to be more dangerous and these types of azo dyes are banned in Germany since 1996 [6], since synthetic dyes, are not ecofriendly. There are several problems associated with synthetic dyes. Hence, people started using cosmetics made with Natural pigments/ dyes [7].

Due to health and Environmental consciousness, most of them prefer natural dyes. Natural dyes are biodegradable and easy to prepare. The demands for natural colorants in cosmetic products are increasing now days. These natural pigments are ecofriendly, non-toxic, non-carcinogenic etc. [8, 9& 10], but their stability, consistency, reproducibility and colour fixation becomes somewhat challengeable. Most of these natural pigments are soluble in water, whereas the synthetic dyes are mostly soluble in oil or other solvents. As these natural pigments are ecofriendly, they have a wide variety of applications in food industry, textile industry, and pharmaceutical industry and also in cosmetics [11].

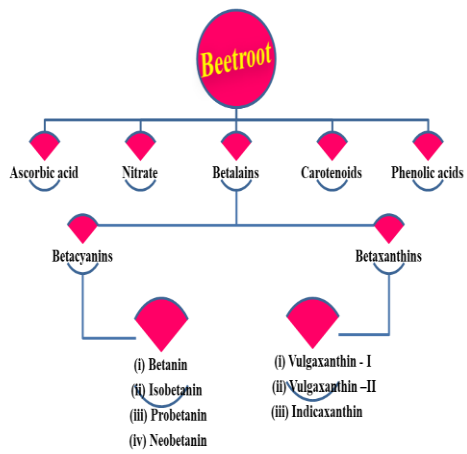

Fig. 1. Overview of the chemical composition in *B. vulgaris*

Red beetroot (*Beta vulgaris* L.) [BV] belonging to family Amaranthaceae are grown in many countries worldwide, is regularly consumed as part of the normal diet, and commonly used in manufacturing as a food colouring agent known as E162 [12,13]. Beetroot is a rich source of phytochemical compounds [14, 15] (Fig. 1). Beetroot is also containing a group of highly bioactive pigments known as betalains [16, 17]. Betalains are water – soluble nitrogen containing tyrosine derived vacuolar pigments [18-19]. BV is a good source of red-violet pigments known as betalains. Betalains consist of betacyanins (red-violet) and betaxanthins (yellow-orange) [20] (Fig. 1). The major betacyanin in beetroot is betanin and accounts for 75–95 % of the red pigment [21, 22]. Betalains can be instantly degraded because of high reactivity under different conditions, such as pH, temperature, oxygen and light [23].

II. MATERIALS AND METHOD

2.1. Chemicals and reagents

HPLC grade water, HCl, NaOH from Merck Specialties Pvt. Ltd., India, were used for the entire study.

2.2. Instruments

Equipment's like Ultraviolet-Visible spectrophotometer (Jasco V-530), Analytical balance (Mettler Toledo), pH meter (Electronic measurement India Pvt Ltd), Water bath, Rotary evaporator and Hot air oven were used.

2.3. Sample collection

Fresh BV were purchased from local market and authenticated with pharmacopoeias and floras. It was washed thoroughly with like warm water to remove the soil and stored at 20°C.

2.4. Extraction of sample

About 100 g of red beetroot were mixed in a blender with 1 liter of ethanol (acidified with 2% citric acid) for 5 min at room temperature and left for 24 hours. The extract was filtered and concentrated under vacuum by a rotary vacuum evaporator at 40º C as reported by Francis [24].

2.5. Preparation of solutions

Exactly 10.0 mg of extracted natural red colour sample was dissolved in 10.0 mL volumetric flask by using HPLC grade water. The absorbance of the samples was scanned from 200 – 800 nm in Ultraviolet-Visible spectrophotometer.

2.6. pH Measurement

The pH of the 0.1 % aqueous solution was determined with the use of a pH meter. The 1000 ppm of extract solution treated with acid and alkali.

2.7. Effect of pH

Sample solution treated with different pH solution with mineral acid, carboxylic acid and alkali. Then treated solution carried out pH and spectrophotometer analysis.

2.8. Effect of temperature

Sample solution was incubated at different temperatures (30º C, 45º C, 60º C, and 75º C respectively) for 30 min and then evaluated for betanin content, based upon maximum absorbance value.

2.9. Effect of photo degradation

The solution were kept with (7,500 – 10,000 lux) and without light surrounding in open and closed container for 24 hrs. Then they were checked for their stability in light and anaerobic conditions, using colorimetric method.

2.10 Spectrophotometric Analysis

The wavelength of the UV-Vis spectrophotometer was set up to 200 nm. Distilled water was used as blank, absorbance were read at intervals of 2 nm wavelength between 200 and 800 nm.

2.11. Betanin content

Content of Betanin in BV were estimated using the formula of Singh et al, [25] as follows:

Total betanin content (mg/g) = A × DF × MW × 1,000/ϵL,

Where, A = absorption value at maximum wavelength, DF = dilution factor, MW = molecular weight of betalain at 550 g/mol, ϵ = extinction coefficient for betalains L = path length of cuvette.

2.12. CIELEB colour point

This is done to prevent different perceptions with persons on specific colors. Hence, an objective way to characterize colors of solution, as well as to quantify differences between colors based on colour point value (L, a*, b*) through UV-Visible system, was evolved [26].

III. RESULTS AND DISCUSSION

3.1 Physio-chemical characteristics and Betanin content

The pH plays an important role in pigment colour degradation, extracted from BV. At low pH upto 3.0, pigment colour degradation was insignificant, whereas, significant degradation in colour were observed at high pH (Fig. 2). Those observations indicated that low and high may not

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be suitable to preserve betanin present in BV (Tab. 1).

Tab. 1. Physio-chemical properties and Betanin content of BV

I.D. No.	Solution with various medium	Colour	pH	Betanin content
1	Extracted solution – as such	Deep red colour	6.5	0.313
2	Weak acid	Almost deep red colour	3.1	0.275
3	Strong acid	Yellowish pink colour	1.8	0.162
4	Strong alkali	Pale yellow colour	11.1	0.057

***** I.D. No. **-** Identification Number

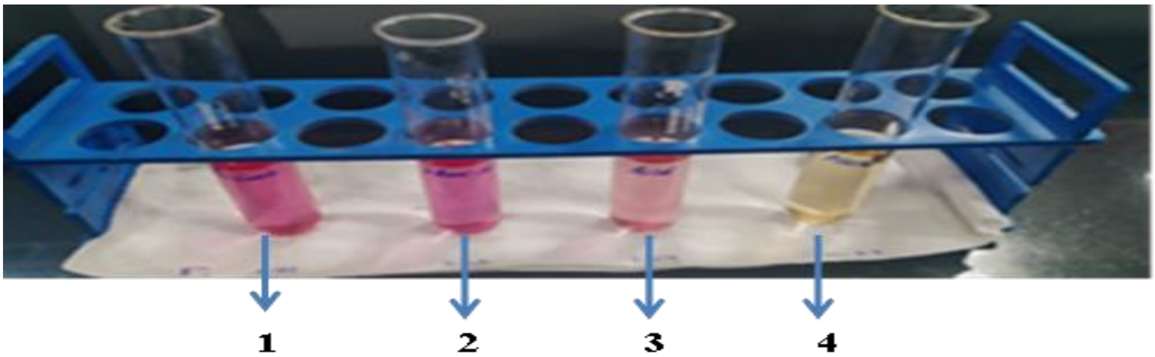

Fig. 2. Natural red colour solution of various pH ranges

3.2. pH

In comparison with various studies carried out on BV colour [27−29], pH ranging from 3 to 7, there was no shift in maximum absorption of betalain (529 nm), and the spectrum were identical without any colour change. However, when the pH is lower than 3, the absorption intensity decreases. At the same time, the absorption degree can be slightly increased, whereas the solution colours were converted from red to purple. When the pH increases (above 9.0), the maximum absorption of wavelength also changes.

Tab. 2. Behaviour of Betanin, natural red colour with various parameters

Sl. No.	**pH range**		**Temperature range**		**Photo degradation**	
	pH	Absorbance	Temperature (ºC)	Absorbance	Light	Absorbance
1	6.5	0.3419	30	0.3419	As such	0.3419
2	3.1	0.3004	45	0.32590	Dark	0.3486
3	1.8	0.1767	60	0.2706	Light	0.3148
4	11.1	0.0621	75	0.2494	Light with air	0.2993

The betalain have a colour spectrum ranging from pink to red, but they are stable at pH 3−7 with optimal stability in the pH range of 4−6 (Fig -1). Present study on pH (6.5; 3.1; 1.8 & 11.0), colour pigments were stable in near neutral pH solution (Tab. 2). Moreover, degradation of colour, does not exceed above 10% between pH 3.0 to 7.0.

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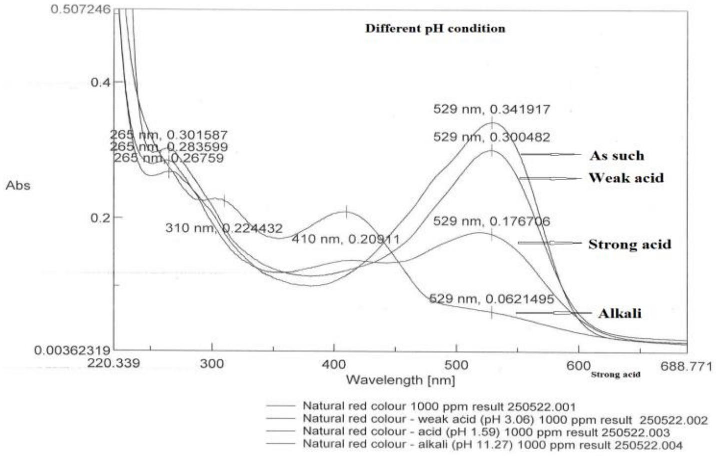

Fig. 3. UV spectrum for different pH ranges in the solution of natural red colour from BV

3.3. Temperature

This is also one of the major crucial factors, towards the stability of betanins, during processing, storage, and shelf life of materials. Betanins are heat sensitive and susceptible to degradation, when the temperature is above 45°C, which is a great challenge, on its application, as food or cosmetics colorant. When, betanin containing solutions are heated, the red colour gradually fades up and turns into yellowish-brown. Degradation of betanin increased gradually by increase in temperature (Fig. 4). The higher degradation rates of betanin were observed above 60° C and the lowest rate was below 50° C. But, on the contrary, at 45° C no significant degradation was observed. Thus the maximum stability of pigment could be in the range of 30° to 45° C (Tab. 2).

In a previous study, it was observed that degradation of betacyanins can occur under high temperatures, forming yellow products, including betaxanthins [30]. Additionally, decarboxylation and elimination of glycoside, can also occur [31].

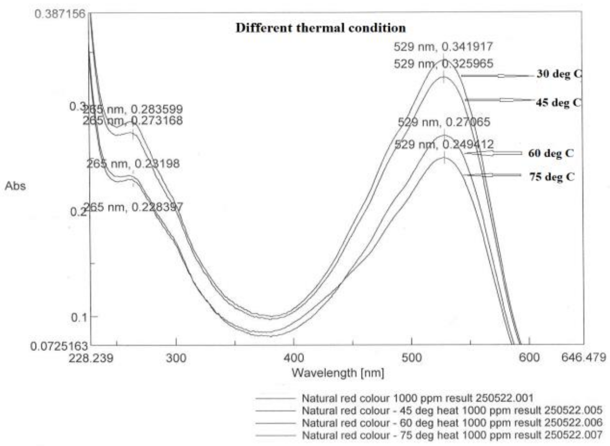

Fig. 4. UV spectrum at various thermal conditions of natural red colour solution for 30 min.

3.4. Effect of photo degradation

The results showed that light could increase the degradation rate for betanin (Fig. 5). The degradation of betanin can be enraged by light and affected to light absorption between ultraviolet and visible spectra, which could promote a transition from an electron excited state to a more active state of the chromophore of betanin [32].

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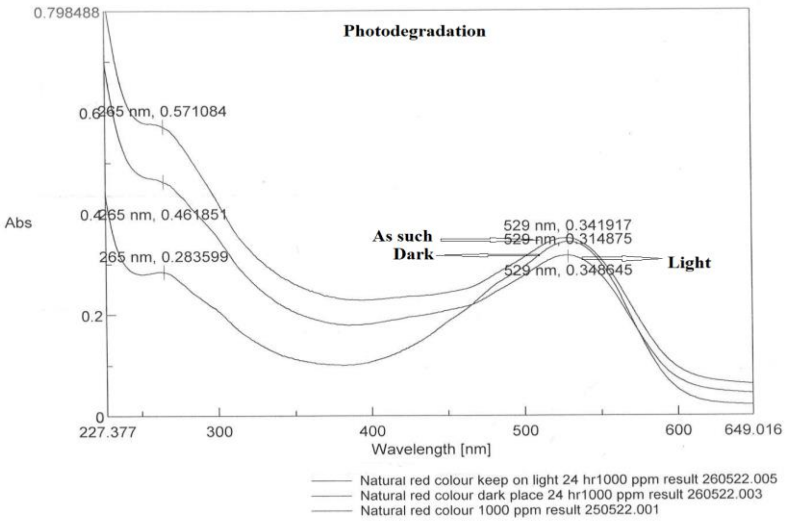

Fig. 5. UV spectrum at various thermal conditions in natural red colour

In addition, light-provoked degradation of betanin is oxygen dependent, since the impacts of light exposure are insignificant under anaerobic conditions (Fig. 6) [33].

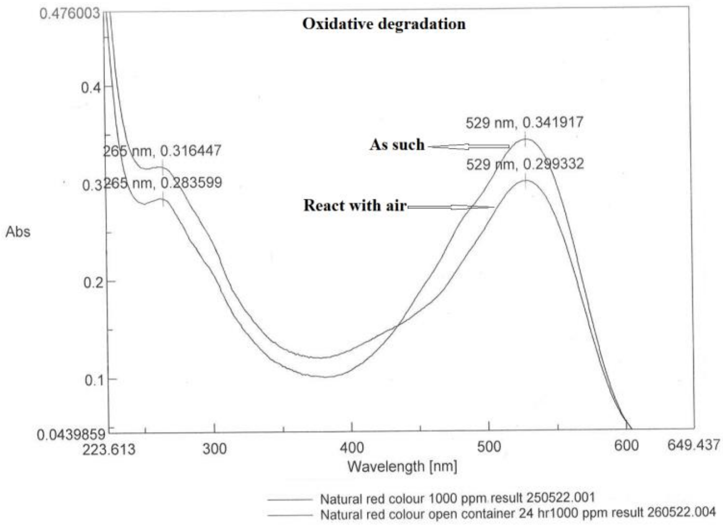

Fig. 6. UV spectrum at various thermal conditions with natural red colour

3.5. CIELAB colour space

The CIELAB colour space, is also referred toas L*a*b*. It expresses colour as three values: L* for perceptual lightness, nd a* and b* for the four unique colours of human vision: red, green, blue, and yellow. It is based upon the opponent colour model of human vision, where red and green form an opponent pair, and blue and yellow form an opponent pair. The lightness value, L*, also referred to as "Lstar," defines black at 0 and white at 100. The* axis is relative to the green-red opponent colours, with negative values toward green and positive values toward red. The b* axis represents the blue–yellow opponents, with negative numbers toward blue and positive toward yellow (Fig. 7, Tab. 3).

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Tab. 3. Photo degradation of betanin due to pH, Temperature and Light intensity

Sl. No.	Different pH range				Temperature range				Photo degradation (24 hrs)			
	pH	L*	a*	b*	Temperature (ºC)	L*	a*	b*	Surrounding (10,000 lux)	L*	a*	b*
1	6.5	79.29	29.19	-0.80	30	79.29	29.19	-0.80	Initial	NA	NA	NA
2	3.1	81.02	25.10	-1.98	45	80.12	27.98	-0.73	Dark	77.01	23.08	2.92
3	1.8	87.10	12.87	2.88	60	83.15	24.19	-0.78	Light	79.51	22.86	2.00
4	11.1	94.30	-1.25	10.62	75	84.32	22.02	0.17	Light with air	81.24	24.94	-0.45

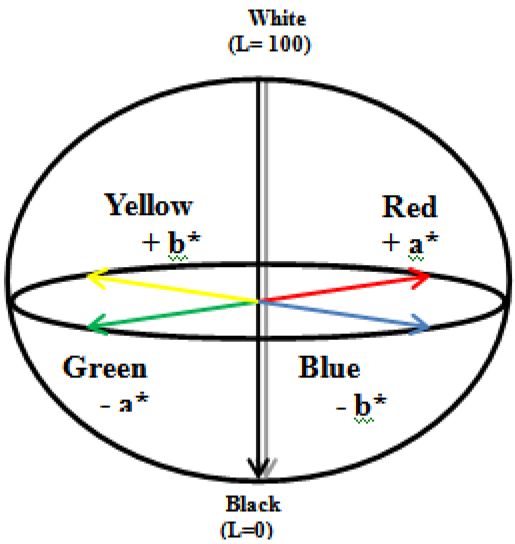

Fig. 7 CIELAB color space diagram

Natural and synthetic colours are compounds of great interest, since they play an important role in our day to day life. On the other hand, synthetic dyes have a lot of important advantages, such as higher stability to light, oxygen and pH, low microbiological contamination risk, long-lasting, cost effective colour uniformity [34-36]. But, due to their non-biodegradable nature, they can affect our ecosystem [37]. Further, synthetic colours, after degradation also can create directly or indirectly, health hazards; which are free with natural dyes [38]. Further, possibility of degradation can be helpful in reducing the usage of synthetic colours in cosmetics and food products.

Colours from natural resources such as vegetables, animals and minerals were popular before the invention of synthetic dyes. Some natural colour such as carotenoids (yellow to red), anthocyanins (red, purple, blue), caramel color (light yellow to dark brown), turmeric (yellow), annatto (yellow to reddish orange) and carmine/cochineal (pink, red, magenta). The carmine found in lipsticks, will not cause harm or health problems when ingested [39].

However, natural colours should be selected with caution, which should be eco-friendly and should also be stable. Since there was no such study on the physiochemical characteristics, the current study was done on the stability and consistency of BV colour with special emphasis to Betanin, which clearly illustrates the wide range of physicochemical conditions to be adhered on the stability of colour with special reference to pH, thermal stability, photo degradation and aerobic condition.

IV. CONCLUSION

This study clearly explains that the stability of Betanin in BV will be stable at a maximum temperature of 40° C and pH should be inbetween 4 to 7. It is also more stable in absence of light source with closed system. Hence these characteristics, will be ideal for those who are using betanin as a colouring agent and fulfilling the criteria which can help in creating a good and viable natural colour.

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