

Effect of extraction conditions on colour content from raw beetroot

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In the present study, a preliminary investigation on extraction and optimization of red colour from raw beetroot has been conducted to optimize the dye extraction process, the extraction parameters, viz temperature is varied from 6 °C to 70 °C, time from 30 min to 120 min and medium pH from 3 to 11. At 45°C temperature, 90 min extraction time and 5 pH, the maximum colour extraction is achieved, i. e. at this optimum condition, the maximum colour yield (2.49 ± 0.08 g/100mL) is obtained. It is observed that at $\geq 47.5^\circ\text{C}$, the chromophore of beetroot dye is denatured as can be seen by the value of λ max, which is changed from 530 nm to 430 nm at $\geq 47.5^\circ\text{C}$.

Keywords: Beetroot, Colour content, Natural dye

Global awareness is favoring the use of natural resources for protecting the environment and earth from pollution and ecological imbalances. The textile industry is one of the biggest polluting industries in the world. In textile chemical processing, a huge number of colorants are used. Most of them are synthetic by nature. Few synthetic colorants are carcinogenic, and their synthesis processes add on to the global carbon footprint. Their ecofriendly substitutes are natural dyes.

In recent years, due to the harmful effect of synthetic dyes (toxicity and carcinogenicity), the interest in natural dyes and colour obtained for colouring of textile, food and cosmetics is increasing continuously¹⁻³. Human skin also demands textile products that are safer and skin friendly, especially for newborn babies and children. Also, many of the synthetic and chemical dyes have a very detrimental effect on human health and at the same time they are not environment friendly^{4,5}. Due to this reason, many countries, like Germany, Holland, France, Turkey,

India, etc. have imposed ban on some synthetic dyes⁶⁻⁸. Textile and other allied industries are in urgent need to explore and discover natural products which can cater the need of natural colour for the industries.

Natural dyes can be sorted into three categories as per source, viz plants based (e.g. Indigo), animals based (e.g. cochineal) and minerals based (e.g. ochre)⁹⁻¹². Natural dyes/colorants obtained from flora and fauna are believed to be safe because of their non-carcinogenic, nontoxic and biodegradable nature¹³. However, use of natural dyes to dye textile materials declined rapidly after synthetic dyes were discovered¹⁴⁻¹⁶. The replacement of natural dyes by synthetic dyes is because, most natural dyes have very poor to moderate light and wash fastness, while synthetic dyes provide the full range of light and wash fastness at moderate costs¹⁷. Lately, however, researchers have started showing greater interest in the revival of natural dyes in textile colouration^{18,19}. This is because of the worldwide concern over toxicity, allergic reactions and carcinogenic effects associated with synthetic dyes^{20,21}. Moreover, many countries have already imposed stringent environmental standards over synthetic dyes. Germany, for instance, imposed ban on the azo dyes which releases banned amines²². In contrast, natural dyes are environmental friendly, exhibit higher compatibility with the environment and show better biodegradability than synthetic dyes²³.

From ancient times the vegetable sources are well known natural colours. The awareness toward environment, health of people, biodegradable nature of the natural colour led to the need of colour extraction from natural resources especially obtained from vegetable dye sources. Till now the natural dye pallet has very few options for red hue. Beetroot is one of the good options for red hue. So, the present study is to focus on the optimization of beetroot dye extraction process extraction by varying extraction conditions, such as temperature, time and medium pH to get maximum yield.

Experimental

Beetroot (*Beta vulgaris*) was procured from the local market for dye extraction. For extraction, RO water was utilised. To set pH, hydrochloric acid

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(procured from Fisher Scientific) and sodium hydroxide (procured from EMPLURA) were used. All chemicals used for different experiments were of laboratory reagent (LR) grade.

Sample Preparation

Thoroughly cleaned and peeled beetroot pieces were manually shredded by mechanical greater for dye extraction. Fine grated beetroot pieces (GBPs) showed average dimensions of $2 \times 5 \times 1$ mm. Fresh samples were prepared before each and every experiment.

Dye Extraction Medium Preparation

For dye extraction purpose, RO water was used as extraction medium. To see the effect of extraction medium pH, aqueous medium of different pH was prepared. The chosen pH range was 3-11. To adjust pH, 1M HCL and 1N NaOH solution were used. Digital pH meter (model no. MK VI, Systronics Ahemdabad) was used to monitor the pH at ambient condition.

Dye Extraction Process

To extract dye, GBPs were submerged into the extraction bath for a preset time at a preset temperature. The detailed recipe is given below:

| Component | Quantity |
|---|----------|
| Dye source (raw beetroot), g | : 5 |
| Extraction medium (RO water), mL [turbidity (NTU scale) – 1, pH~ 8.4] and total dissolved solid (TDS) – 90-150 ppm] | : 50 |
| Material - to - liquor ratio | : 1:10 |
| Temperature, °C | : 6-70 |
| Time, min | : 30-120 |
| pH | : 3-11 |

To observe the effect of different extraction medium parameters on dye extraction, each and every parameter was varied, while keeping other at constant. For example, the first medium parameter was temperature. To check its effect on dye extraction, medium temperature was varied from 6 °C to 70 °C, while maintaining the medium pH at 7 and time as 120 min. Further parameter variations were done where the process temperature was kept constant at which maximum yield was obtained from previous experiments.

The effect of every parameter was assessed based on colour yield. Visible spectrophotometer (Model - SS5100A) was used to measure colour yield with

respect to standard calibration plot. Beetroot powder (BRP) was used to prepare standard calibration plot. Standard colour samples were prepared by extracting colour from BRPs in dye extraction medium of pH 7 by keeping temperature at $25 \pm 2^\circ\text{C}$ for 120 min. Details of the process parameters are shown below:

| Component | Quantity |
|--|--------------|
| Dye source (raw beetroot), g | : 5 |
| Extraction medium (RO water), mL [turbidity (NTU scale) – 1, pH~ 8.4] and total dissolved solid (TDS) – 90-150ppm] | : 50 |
| Material - to - liquor ratio | : 1:10 |
| Temperature, °C | : 25 ± 2 |
| Time, min | : 120 |
| pH | : 7 |

Serial dilution process was acquired to prepare six samples of known concentration from 10% BRP stock solution. Absorbance value of dye solution at different concentrations after serial dilution is depicted in Table 1 and calibration plot between concentration and absorbance value is shown in Fig. 1.

Absorbency of all samples were measured to obtain relationship between beetroot concentration (X) and absorbance value (Y), which is given below:

$$Y = 3.498X \quad \dots(1)$$

Assessment of all the experiments and findings was done on the basis of Eq. (1). In this research, all the experiments are repeated at least for 5 times. Exceptional cases are mentioned separately.

Table 1 — Absorbance value of dye solution at different concentrations after serial dilution

| Concentration, % | Absorbance value, AU |
|------------------|----------------------|
| 0 | 0 |
| 0.1 | 0.03 |
| 0.2 | 0.07 |
| 0.3 | 0.11 |
| 0.4 | 0.14 |
| 0.5 | 0.18 |

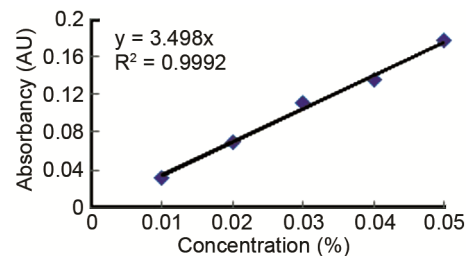


Fig. 1 — Calibration plot between concentration and absorbance value

Results and Discussion

The biggest challenge faced from natural dyestuff application is the limited available sources for red shades. Beet root is very well known for its red colour. The pigment in beetroot, which is responsible for the red colour, is betanin²⁴⁻²⁶. Betanin is very well known for its red colour and mostly used in food and pharmaceutical products as a natural red colour, but its use in textile field is limited. Betanin group of pigments contains both yellow and red pigments known as Betacyanins and Betaxanthins respectively^{27,28}. In the visible spectrum beetroot extract gives red hue at 530nm wavelength and golden yellow hue at 430nm. Beetroot colour is well established as food colour agent for putting red hue^{29,30}, but for fabric dyeing, optimizing process has not been reported yet. So, we try to optimize the beetroot extraction process using different parameters, like extraction temperature, time and medium pH.

Effect of Temperature on Dye Extraction

In this study, the temperature of the dye extraction system is varied between 6 °C and 70 °C for 2 h at 7 pH. For heating the dye extraction system hot plate has been used for different range of temperature (25 - 70 °C). To attain 6 °C, extraction medium and beetroot both are kept at 6°C in the refrigerator (Model SBS 560W, MARQ) for 2 h. Afterwards, the sample is added in the extraction medium (i.e. RO water) at 6°C for 2 h. The absorbance value of all the samples is measured through a spectrophotometer (Model – SS5100A) and concentration yield is calculated using Eq. (1) and given in Table 2.

From the experimental results of the extraction carried out at different temperatures keeping pH 7 and extraction time 2h, 45°C temperature is the optimum temperature at which maximum extraction of beetroot dye from raw beetroot is found. This is because, at this temperature, absorbance value as well as average

Table 2 — Average concentration yield of extracted dye at various temperature, keeping 7 pH and 2 h time

| Temperature, °C | λ_{\max} , nm | Avg. yield, g/100mL |
|-----------------|-----------------------|---------------------|
| 6 | 530 | 1.5975 ± 0.06 |
| 25 | 530 | 1.7741 ± 0.04 |
| 32 | 530 | 1.4888 ± 0.05 |
| 40 | 530 | 2.0841 ± 0.11 |
| 45 | 530 | 2.5677 ± 0.13 |
| 47.5 | 430 | 1.3951 ± 0.19 |
| 50 | 430 | 1.2748 ± 0.10 |
| 60 | 430 | 1.3813 ± 0.08 |
| 70 | 430 | 1.4951 ± 0.08 |

concentration yield are maximum. Other observation is that after 47.5°C, the chromophore of beetroot dye is denatured, as can be seen by λ_{\max} value, which is changed from 530 nm to 430 nm after 47.5°C.

Effect of Time on Dye Extraction

In this study, the time of the dye extraction system is varied between 30min and 120 min at the optimized temperature 45°C and 7 pH, the absorbance value of all the samples is measured through spectrophotometer (Model – SS5100A) and concentration yield is calculated through the Eq. (1) and given in Table 3.

From the experimental results of the extraction carried out at 45 °C temperatures, pH 7 and keeping different extraction time duration, 90 min is found to be the optimum extraction time at which maximum extraction of beetroot dye from raw beetroot is done, as at this time duration absorbance value as well as average concentration yield are maximum.

Effect of pH on Dye Extraction

In this study, the medium pH of the dye extraction system is varied from 3 pH to 11 pH at the optimized temperature 45 °C and for optimized time 90 min. The absorbance value of all the samples is measured through a spectrophotometer (Model – SS5100A) and concentration yield is calculated through the Eq. (1) and given in Table 4.

From the experimental results of the extraction carried out at 45 °C temperatures for 90 min keeping different extraction medium pH, 5pH is the optimum value at which maximum extraction of beetroot dye from raw beetroot is done, as at this pH absorbance value as well as average concentration yield are maximum.

Table 3 — Average concentration yield of dye extracted at various time span, keeping temp. at 45 °C and pH 7

| Time, min | λ_{\max} , nm | Avg. yield, g/100mL |
|-----------|-----------------------|---------------------|
| 30 | 530 | 1.6541 ± 0.34 |
| 60 | 530 | 1.8092 ± 0.32 |
| 90 | 530 | 1.9398 ± 0.17 |
| 120 | 530 | 1.6378 ± 0.43 |

Table 4 — Average concentration yield of dye extracted at various pH, keeping temp. at 45 °C and time 90 min

| pH | λ_{\max} , nm | Avg. yield, g/100mL |
|----|-----------------------|---------------------|
| 3 | 530 | 2.3962 ± 0.08 |
| 5 | 530 | 2.4934 ± 0.08 |
| 7 | 530 | 2.2567 ± 0.03 |
| 9 | 530 | 2.1366 ± 0.05 |
| 11 | 430 | 2.1886 ± 0.04 |

From the above study, it is concluded that there is a prominent effect of extraction conditions, viz. extraction temperature, time and pH on the extraction of colour content from the beetroot. It is found that, 45 °C temperature, 90 min extraction time and 5 pH are the optimum conditions for maximum beetroot dye extraction. Optimum extraction condition results in maximum beetroot dye extraction with a yield concentration of 2.49 ± 0.08 g/100mL (w/v). However, after 47.5 °C, the chromophore of beetroot dye is denatured. Betanin denaturization is confirmed by the change in λ_{max} from 530 nm to 430 nm. The extracted beetroot dye is intended to colour different natural (protein and cellulosic) fibres, like wool, silk, cotton, etc.

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