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# Isolation and Stabilization of Dark Red Food Dye from *Beta vulgaris*

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**Abstract.** Natural highly coloured dark red pigment was isolated from *Beta vulgaris*, in paste and powdered form. Total colouring matter of the concentrated colour was 1.86% and 4.5%, respectively, for the paste and powdered forms, calculated as betanine. Sodium benzoate (0.01%) was used as the stabilizer for paste, while silicon dioxide (2%) was added in addition to sodiun benzoate (0.01%) for storage of the red colour in powdered form. Other parameters that may influence the stability of the colour, such as pH, temperature and relative humidity, were studied. Toxicity evaluation, and lead and arsenic levels were determined. The addition of stabilizers, like citric acid, ascorbic acid, EDTA and sodium chloride, were also investigated, none of which showed useful effect.

**Keywords:** food colour, betanine, beetroot colour, *Beta vulgaris* red colour, food colour isolation, food colour stabilization

## Introduction

Beetroot contains betenine ($C_{24}H_{26}N_2O_{13}$) as the principal pigment, which is the D-glucopyronoside of betanidine (FAO, 1984). It is obtained from the roots of red beet (*Beta vulgaris*). The red colour of beetroot is suitable for products having relatively short shelf-lives and where the food stuff has not to undergo high or prolonged heat treatment (Coulson, 1980). Stability is higher in the pH range of 4-5, though the stability is reasonable in the pH range of 3-7. Adding colour, after the heating process has ceased, can be successfully done for the colouration of foods that have to undergo heat treatment. Adding small amount of benzoate, sorbate or EDTA can enhance stability of the colour. Beetroot red colour may be used, with the addition of a suitable stabilizer, in soft drinks, ice cream, meat and soyabeen protein products, and in dry mixes such as gelatin desserts (Coulson, 1980).

Various laboratory techniques have been reported for the isolation of colour from beetroot (Krasnikova *et al.*, 1996). The rich red dye in powdered form has been obtained by heating beetroots at 100 °C for 5 min. The peroxide present can be inactivated, and the colour extracted with aqueous citric acid (Lozano *et al.*, 1993). The red dye has been also obtained by the aqueous extraction of colour from beetroots (Kutsakova *et al.*, 1997). Red dye from beet was earlier obtained by preheating beet slices with 0.125% citric acid at 100 °C for 20 min and the average recovery was reported as 63.3% (Liu, 1981).

The presently developed procedure involves the extraction of red food dye from beetroots with salicylic acid (0.125%), which was then concentrated by freeze drying. The purpose of the present study was to produce natural red dye in powdered as well as in paste form, to enhance the stability of colour at different pH values and temperatures, to study any toxicological effect of the extracted red food dye, and to determine the usefulness of the obtained colour in different foodstuffs. The principal objective of the present study was to produce natural food dye that is non-toxic and harmless to human health, since it has been claimed that several artificial colours and flavours used in foods may lead to hyperactivity and learning disability in children (Feingold, 1975). It was estimated that 50% of the hyperactive children could be completely cured by a diet, totally devoid of these chemicals.

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## Materials and Methods

**Instruments used.** Freeze dryer (Eyela; FD 550), vacuum oven (Hitachi Yamato; DP 41), hotplate blender (Waring), spectrophotometer (Hitachi; U-1100), atomic absorption spectrometer (Hitachi, 170-10).

**Chemicals.** Sodium benzoate (Win Laboratories); citric acid, salicylic acid, silicon dioxide, ascorbic acid, disodium hydrogen phosphate (E. Merck, Germany); EDTA (BDH).

**Extraction of the colour.** The red colour was extracted with 0.125% solution of salicylic acid by blending fine slices of beetroots in a Waring blender at medium speed for 5 min. The extract was filtered through Whatman filter paper# 1. Residues were washed thrice with salicylic acid solution.

**Drying techniques.** Various drying techniques were used to concentrate the fresh colour extracted from the beetroots, such as waterbath drying, hotplate drying, sun-drying, vacuum oven-

drying, and freeze drying. Maximum betanine contents (the colouring matter of beetroots) were obtained when the freeze drying method was used. The colour dried with other techniques resulted in the product having lesser colour content, which turned reddish brown. Therefore, the freeze drying technique was used to concentrate the extracted red colour.

**Freeze drying technique.** The extracted colour was dried in a freeze dryer in two steps. In the first step, the extracted red colour was frozen and the temperature of the freeze dryer was set according to the quantity of the extract (if volume of the sample was less, then it was frozen at - 20 °C; if the quantity of the sample was greater, then the temperature was lowered down, according to the requirement). In the second step, sublimation was initiated, and the dry mass was obtained within 6 to 8 h.

**Measurement of colour contents.** The total colour contents were measured spectrophotometrically in McIlvaine's citric acid buffer (pH 5) by measuring absorbance at 535 nm (FAO, 1984). The colour intensity was calculated on the basis of maximum absorption. All the red colouring matter is betanine, which was calculated as below (FAO, 1984):

absorbance × 100/concentration × A [1%, 1cm]

**Metallic impurities.** The extracted colour was digested in a mixture of sulphuric acid, nitric acid and perchloric acid. The digested metals were determined by atomic absorption spectroscopy according to the method of FAO (1984).

## Results and Discussion

**Percentage of colour contents.** The colour obtained after freeze drying was stored in the paste and powdered forms. Total colour contents were 4.5% in the powdered form and 1.86% in the paste form. Higher percentage of betanine was obtained due to the lesser degradation of betanine in the selected drying technique.

**Effect of relative humidity.** Relative humidity was noted to influence colour contents, as well as appearance of the colour, during storage (Table 1). It is evident from these results that the powdered form exists only upto 40% relative humidity and colour contents were also higher at this relative humidity. However, with increase in relative humidity (60%), the colour intensity slightly decreased and the extracted red colour was converted to a hard mass. At 70% relative humidity and above, the colour contents decreased to 1.86% and the colour was converted to the paste form. Stringent control of conditions, like humidity and temperature, is required to maintain colour in powdered form. Silicon dioxide was also added in the extracted red colour to avoid settling of the colour, which was also observed to enhance shelf-life of the colour and for maintaining it in the powdered form for a longer period of time.

**Table 1.** Effect of relative humidity on the stability and appearance of the red colour extracted from beetroots, when stored at 25 °C for a period of six months

| Relative humidity (%) | Colour* contents (%) | Appearance of the colour |
|---|---|---|
| 30 | 4.5 | powder |
| 40 | 4.3 | setteled powder |
| 60 | 4.0 | hard Mass |
| 70 | 2.0 | paste |
| 80 | 1.86 | paste |

* = measured as betanine

**Effect of stabilizers.** The effect of different stabilizers on the stability of the extracted colour is shown in Table 2. Stabilizers were used individually and in different combinations. It was found that intensity of the colour, without any stabilizing chemical, decreased within few weeks. It was observed that addition of sodium benzoate (0.01%) to the colour extracted with salicylic acid (0.125%) significantly enhanced the stability as well as shelf-life of the colour, and the assayable content as betanine remained constant for upto one year. Intensity and stability of the colour has been reported to be enhanced on the addition of ascorbic acid (0.125%) with sodium benzoate (0.01%), and citric acid (0.125%) with sodium benzoate (0.1%) (Krasnikova *et al.*, 1996). Similar observation was made by Chorbanov *et al.* (1988) and Zherebin *et al.* (1991). However, it was concluded that shelf-life and total colouring matter decreased when sodium benzoate was added to the extracted colour as stabilizer. Colour with sodium chloride as the stabilizer, absorbs moisture and turned to liquid form even after 24 h of drying. EDTA (0.1%) also increased the stability, but sodium benzoate was noted to be a better stabilizer than EDTA. It was also concluded that the colour stored in powdered form settled into a hard mass after few months. Silicon dioxide (2%) was added to overcome this problem.

**Effect of pH.** The colour isolated from beetroots showed maximum stability in the pH range of 4.5-7.5 (Fig. 1). At the lower pH (2.5) level, reduction in the colour contents was noted as the colour turned to light red. At pH values higher than 7.0, the colour changed from dark red to reddish violet, which turned to yellow towards the higher pH values, upto pH 12.0. Similar reports have been published by other researchers that betalains are especially stable at pH 5.0, which could be stabilized against decolourizing and the action of antioxidants

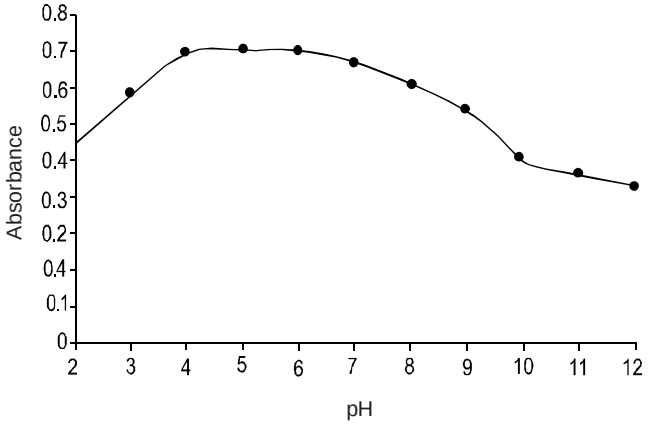


**Fig. 1.** Effect of pH on the stability of red colour extracted from beetroots.

by acidifying their solutions to pH 4.5 to 5.0 (Hamburg and Hamburg, 1991).

**Effect of storage temperature.** Colour was stored at different temperatures and best results were obtained at 15-35 ˚C (Table. 3). Above this temperature, the assayable contents reduced and the colour became unstable, which may be due to the degradation of the main colouring component (betanine) at the higher temperature. It was observed that at lower temperature, there was no remarkable change in the colour during storage period of one year. However, at higher temperature (60 °C), the storage period induced a drastic change in the assayable contents of the colour. There was no apparent trend of colour change at lower temperatures, suggesting that the colour remained stable at lower temperature during storage.

**Table 2.** Effect of different stabilizers on the stability of colour extracted from beetroots

| Stabilizers (%) | Colour contents (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sep 2002 | Oct 2002 | Nov 2002 | Dec 2002 | Jan 2003 | Feb 2003 | Mar 2003 | Apr 2003 | May 2003 | June 2003 | July 2003 |
| Sodium benzoate (0.01)* | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.78 | 1.76 | 1.75 | 1.70 |
| Sodium benzoate (0.01) + ascorbic acid (0.125)* | 1.70 | 1.70 | 1.68 | 1.65 | 1.50 | 1.30 | 1.15 | 1.00 | 0.85 | 0.70 | 0.63 |
| Sodium benzoate (0.01) + citric acid (0.125)* | 1.60 | 1.60 | 1.50 | 1.35 | 1.32 | 1.25 | 1.22 | 1.00 | 0.80 | 0.68 | 0.50 |
| Sodium chloride (0.5)* | 0.9 | 0.63 | 0.50 | 0.28 | 0.15 | 0.10 | 0.05 | - | - | - | - |
| EDTA (0.1)* | 1.25 | 1.25 | 1.23 | 1.23 | 1.20 | 1.18 | 1.15 | 1.12 | 1.10 | 1.10 | 1.09 |
| Sodium benzoate (0.01) + silicon dioxide (2.0)** | 4.50 | 4.50 | 4.50 | 4.45 | 4.50 | 4.50 | 4.50 | 4.48 | 4.45 | 4.43 | 4.40 |

* = paste form; ** = powder form

**Table 3.** Effect of storage temperature on the stability of colour extracted from beetroots

| Storage temperature (°C) | | Colour contents (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sep 2002 | Oct 2002 | Nov 2002 | Dec 2002 | Jan 2003 | Feb 2003 | Mar 2003 | Apr 2003 | May 2003 | June 2003 | July 2003 |
| 15 | paste | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.85 | 1.83 |
| | powder | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.43 | 4.40 | 4.38 | 4.35 |
| 25 | paste | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.84 | 1.82 | 1.80 | 1.80 | 1.79 |
| | powder | 4.50 | 4.50 | 4.50 | 4.50 | 4.45 | 4.42 | 4.42 | 4.40 | 4.35 | 4.35 | 4.30 |
| 35 | paste | 1.86 | 1.86 | 1.86 | 1.85 | 1.85 | 1.85 | 1.80 | 1.78 | 1.75 | 1.71 | 1.69 |
| | powder | 4.50 | 4.45 | 4.43 | 4.40 | 4.40 | 4.38 | 4.35 | 4.32 | 4.30 | 4.25 | 4.00 |
| 60 | paste | 1.86 | 0.75 | 0.50 | 0.18 | 0.05 | - | - | - | - | - | - |
| | powder | 4.45 | 3.20 | 2.58 | 2.02 | 1.16 | 1.02 | 0.95 | 0.81 | 0.55 | 0.35 | 0.30 |

**Toxicity and heavy metals.** Toxicity test was performed on mice and it was observed that the extracted colour was non-toxic. Lead and arsenic were not detected in the extracted colour, while other heavy metals were found within the limits specified by FAO (1984).

**Stability of colour during cooking.** The effect of cooking time and temperature is shown in Fig. 2. It is evident that the colour remained stable upto 80 °C for 15 min. Above this time and temperature, colour intensity decreased and colour became unstable.

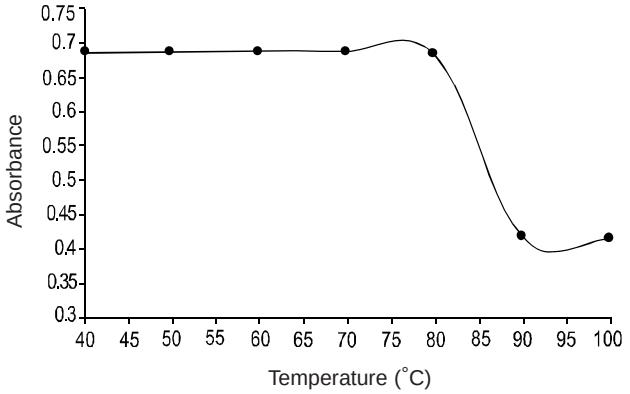


**Fig. 2.** Effect of temperature on the stability of colour extracted from beetroots during 15 min cooking; conditions: pH (4.0), stabilizer (sodium benzoate, 0.01%).

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