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# Betalain – a boon to the food industry

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**ABSTRACT**

Colour is one of the most important quality attributes affecting the consumer's acceptance of food. Natural colors are pigments made by living organisms and the three most important being tetrapyrrols, tetraterpenoids and flavonoids. The terms "pigment" and "dye" are often used interchangeably. Strictly speaking, pigments are insoluble in the given medium, whereas dye is soluble. Betanin is the main coloring compound present in red beetroot juice color. Historically, it has imparted additional color to wines. The colourings responsible for the red hue of red beet juice are a group of molecules called *betalains*. This group of pigments contains the red and yellow pigments known as *betacyanins* and *betaxanthins*, respectively. Beets contain a significant amount of vitamins A and C and also calcium, iron, phosphorus, potassium, protein and carbohydrates. They are also high in folate, dietary fiber and antioxidants and have high betaine which is used to lower toxic levels of homocysteine (Hcy) which contributes to the development of heart disease, stoke and peripheral vascular disease. The present study comprises of extraction of betalain pigment followed by Phytochemical Analysis, Estimation of Flavonoids and Evaluation of Antioxidant capacity by Phosphomolybdenum method. Stability of the Betalain pigment was checked against Temperature and pH and the pigment was purified using Column Chromatography and confirmed by Thin Layer Chromatography. The pigment was finally applied to a natural food to test its significance.

**Keywords:** Natural Colour, Beets, Betalain pigment, *Beta vulgaris* and Colouring Dye.

## 1. INTRODUCTION

The most important member of the tetrapyrrols is chlorophyll, which is found in all higher plants. Carotenoids are tetraterpenoids that are as ubiquitous as color as chlorophyll, since they too are part of the photosynthetic apparatus (Kalyani et al., 2008). They also

give the yellow–orange–red color of color to many fruits. Anthocyanins are a group of flavonoids which provide the red–purple shade of many fruits, particular berries (e.g., strawberries, elderberries and black currants). Other important classes of colorants are anthraquinones (carmine, lac, kermes and madder) and the betalains (beetroot) (Bor-Yann Chen et al., 2007).

*Beta vulgaris* is a herbaceous biennial or, rarely, perennial plant with leafy stems growing to 1–2 meters tall. It comes under the family Chenopodiaceae commonly called as table beet, garden beet, red or golden beet, or informally as beet (Strack et al., 2003). The leaves are heart-shaped, 5–20 cm long on wild plants**.** The flowers are produced in dense spikes; each flower is very small, 3–5 mm diameter, green or tinged reddish, with five petals; they are wind pollinated. The fruit is a cluster of hard nutlets (Cai et al., 2001). They are mainly grown for their edible taproots. However, other cultivated varieties such as the leaf vegetable chard, as well as the root vegetable sugar beet is used for production of table sugar, and mangelwurzel, as a fodder crop (Bilyk and Howard, 1982). Three recognized subspecies include *Beta vulgaris* subsp. *vulgaris*, *Beta vulgaris* subsp. *maritima*, known as the sea beet found throughout the Mediterranean, the Atlantic coast of Europe, and Kashmir and *Beta vulgaris* subsp.*adanensis*, occurs from Greece to Syria (Azeredo, 2009). The roots are most commonly deep red-purple in color, but come in a wide variety of other shades, including golden yellow and red-and-white striped (Figure 1).

Beets are readily available for food and beverage manufacturers and are available in two primary forms: Ground Dehydrated Beets the dehydrated beet vegetable which is ground into a powder and Beet Juice the juice from the red beet, which can also be spray dried into powder form. Betalains do have excellent light stability and excellent pH stability (Chethana et. al., 2007). Generally, betalain colors will not fade in light (Gonçalves et al., 2013). Unlike anthocyanins, betalains do not change in hue in response to differences in the pH of foods and beverages. The have enormous medical applications (Bartoloni et al., 2013). Betalains guard the slim lining of one's blood vessels and helps to reduce the inflammation that makes the blood sticky and results in clots. It reduces bad cholesterol and strongly reduce oxidized LDL cholesterol (Figure 2). It protects cells from toxins and guards various types of cells, particularly brain cells, from harmful toxins known to trigger tumors. It protects the liver and provides important protection from toxins that directly impact on your liver. Limitless to say it guards the body against unsafe toxins that threaten cellular health, result in inflammation as well as trigger a whole host of diseases (Gonçalves et al., 2013).

The stability of betalains dictates their range of food colouring applications. Betalain extracts need to be treated with care because they are sensitive to environmental conditions, particularly pH, heat, light, moisture and oxygen (Delgado-Vargas et al., 2000). These environmental factors can cause discoloration of the pigments under adverse conditions. The red pigment betanin, for example, degrades on exposure to air, bright light and high temperatures to a light brown colour. This discolouration is partially reversible, if adverse conditions are only temporary (Degenhardt and Winterhalter, 2001).

Betalain colouration is unaffected by pH in the range 3.5 to 7.0 (acid to neutral). Beetroot extracts in most foods will therefore not discolor as a direct result of pH (Fulcrand et al., 1998). The optimum pH for both betacyanin and betaxanthin pigments occurs in the slightly acidic 5.0 - 6.0 range**.** The colour of red beetroot extract changes from red towards blue as pH increases above 7.0. Root tissue exposed to high or alkaline pH (7.5 - 8.5) becomes discolored. Cut beetroot retains its purple-red colour well in acidic solutions such as malt vinegar (acetic acid)**.** The juice is good for blisters and blains of the skin and as a decoction in water and vinegar cleanses the dandruff and relieves running sores and ulcers. Red beet juice is used to cure yellow jaundice and when the juice is put in the nostrils, it is helpful for ringing in the ears and toothaches. Uridine isolated from sugar beets can be used along with omega-3 to alleviate depression. It is an effective detoxifier and also used in the treatment of AIDS**.** Beetroot pigment is used commercially as a food dye. It changes colour when heated so it is used only in ice-cream, sweets and other confectionary, but it is both cheap and has no known allergic side-effects. The use of bio-colorants may show benefits over synthetic colours. Natural dyes are less toxic, less polluting, less health hazardous, non-carcinogenic and non-poisonous and prevent chronic diseases such as prostrate cancer. In addition to this, they are harmonizing colours, gentle, soft and subtle, and create a restful effect. Most of them are water-soluble which facilitates their incorporation into aqueous food systems. These qualities make natural food colorants attractive. Above all, they are environment friendly and can be recycled after use. Thus, they attribute to food-both for aesthetic value and for quality judgment and also they tend to yield potential positive health effects, as they possess potent antioxidant and improve visual acuity properties (Gaertner and Goldman, 2005).

## 2. MATERIALS AND METHODS

### 2.1. Extraction of Betalain and Anthocyanin

Aqueous ethanol (50:50) -100mL, Beet root fruit (peeled) -100g were the materials required. Extraction of pigment was achieved by homogenization of equal ratio of sample and solvents (1/1 w/v). 100 g of the peeled fruit of beet root was weighed and macerated with 100 ml solvents (EtOH, aqueous ethanol 50:50) for 15 minutes. The aqueous mixture was centrifuged at 12,000 rpm at 4˚C for

20 min. The supernatant was taken and concentrated using rocker. The ethanol was removed after concentration process and samples were kept in a dark bottle.

**Figure 1**
*Beta vulgaris*

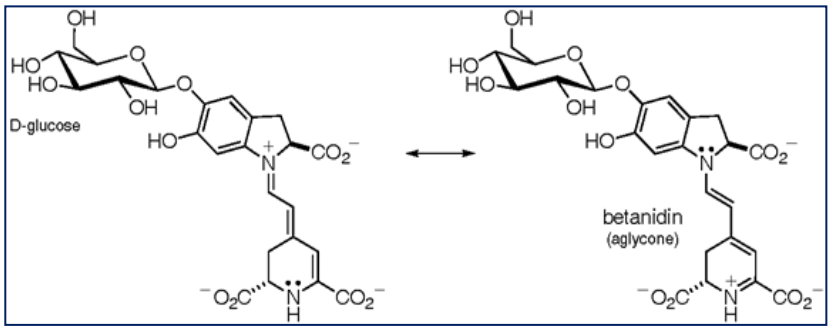



**Figure 2**
Structure of Betalain

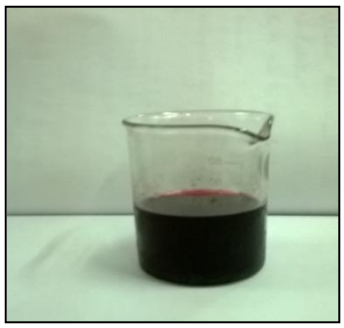


**Figure 3**
Betalain extracted from *beta vulgaris*

**Figure 4**
Phytochemical analysis of beetroot (tannins)

**Figure 5**
Saponins

**Figure 6**
Flavonoids

**Figure 7**
Reducing sugar

**Figure 8**
Volatile oilterpenoids

## 2.2. Phytochemical Analysis of Betalain Extract

To 2ml of the extract, 2ml of distilled water was added followed by few drops 1% lead acetate. Formation of white precipitate indicates the presence of tannins. 1ml of the extract was shaken vigorously with 1ml of distilled water in a test tube and warmed. The formation of stable foam, honey comb like shapes indicates the presence of saponins. To 2ml of the extract few drops of acetone was added .It was then kept in water bath to evaporate acetone. After evaporation boiling water was added and then it was cooled .It was followed by the addition of 5ml of 20% NaOH. Appearance of yellow colouration indicates the presence of flavonoids. To 0.5ml of the extract 1ml of distilled water and 5-8 drops of fehlings solution was added and heated over water bath. Appearance of brick red indicates the presence of reducing sugars. To 2ml of the extract 0.1ml of NaOH was added and shaken vigorously followed by addition of small amount of distilled water. Formation of white precipitate indicates the presence of volatile oil. 5ml of

each extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

### 2.3. Estimation of Flavonoids

5% Sodium Nitrite, 10% Aluminium Chloride, Sodium Hydroxide, Distilled water and Volumetric flask were the materials required. An aliquot (1 ml) of extract (concentration 1 mg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled $H_2O$. To this 0.3 ml 5% $NaNO_2$ was added and 5 minutes later 0.3 ml 10% $AlCl_3$ was added. After 6 minutes, 2 ml of 1M NaOH solution was added. Finally the total volume was made up to 10 ml with distilled $H_2O$. The solution was well mixed. The absorbance was measured at 510 nm against the control. Control was prepared in the same manner only with replacing the extract with distilled water.

### 2.4. Evaluation of Antioxidant Capacity by Phosphomolybdenum Method

Test tubes, Colorimeter, 0.6M Sulphuric acid, 28mM Sodium phosphate, 4mM Ammonium molybdate and Distilled water were the required materials. An aliquot of 0.3 ml of sample solution was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in colorimeter. A typical blank solution containing 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample was incubated under same conditions as rest of the sample. For samples of unknown composition, water-soluble antioxidant capacity was expressed as equivalents of ascorbic acid.

### 2.5. Stability to Temperature

Heat stability was evaluated by exposing the color extracts to different temperatures such as 40°C, 50°C, 60°C 70°C, and 80°C for different time periods of 5, 10, 15 and 20 minutes. After every treatment the extract were immediately cooled. The absorbance of beet root extract was read at 537nm.

### 2.6. Stability to PH

HCl -0.1M and NaOH -0.1M were the reagents required. The pH of the extracts was adjusted using 0.1M HCl and 0.1M NaOH. The pH ranges of 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 were attained. The absorbance of beet root extract was read at 537nm.

### 2.7. Purification of Pigments Using Column Chromatography

When a mixture of mobile phase and sample to be separated are introduced from top of the column, the individual components of mixture move with different rates. Those with lower affinity and adsorption to stationary phase move faster and eluted out first while those with greater adsorption affinity move or travel slower and get eluted out last. A vertical glass column with a knob at the bottom end, cotton, Silica gel (120 mesh), Solvent (Ethanol: Water – 8: 2) and sample were the materials required. The betalain extracted using ethanol was concentrated using rocker before purification. A cylindrical glass column was taken and was plugged with a small piece of cotton. The column was mounted on the stand. 25g of fresh silica gel (120 mesh) was taken in a 250 ml beaker. 100 ml of solvent was poured into the beaker and stirred well using a glass rod to make slurry of the silica. The slurry was poured into the column. The conical flask was placed below the mounted column and the excess solvent was drained out. The solvent was eluted for few times until the column gets well packed. The knob was closed when the level of the solvent reached just above the settled silica gel. The sample was transferred into the solvent layer above the silica gel in the packed column. The column was continually filled with ethanol and was eluted until the pigment runs down the column. The elution was performed with the same binary solvent mixture at a flow rate of 0.7 mL/min. As the elution progresses the pigment elutes out of the column and was collected in a conical flask. The silica gel 60 column was not regenerated. The pigments collected from the column are then concentrated by removing the solvents using rocker. The pigments left behind in the round bottomed flask after concentrating are transferred and stored.

**Table 1**

| PHYTOCHEMICAL TEST | BEET ROOT (*Beta vulgaris*) |
|---|---|
| TANNINS | + |
| SAPONINS | + |
| FLAVONOIDS | + |

| | |
|---|---|
| REDUCING SUGARS | + |
| VOLATILE OIL | - |
| TERPENOIDS | + |

### 2.8. Confirmatory test for Betalain Pigments

**Thin Layer Chromatography**

TLC works on the principle of capillary action. Separation occurs as each component, being different in chemical and physical composition, interacts with the stationary and mobile phases to a different degree, creating the individual bands on the plate. The retardation factor, $R_f$ value, is used to characterize and compare components of various samples.TLC plates, capillary tubes, beaker, sample, Solvents - 1% Hcl for Betacyanin were the materials required. A TLC plate was taken and with a pencil a line was drawn approximately 1 cm from the short edge of the TLC plate. Care was taken not to scrape the coating of the plate. With a capillary tube, the sample was spotted on the TLC plate. The spot was labeled at the top of the TLC plate. The sample was reapplied to the same place at least 3 times or until the spot is clearly visible. The chromatography chamber was filled to a depth of approximately 0.5 cm with the 1% aqueous HCl for betacyanin pigment. The TLC plate was placed in the chromatography chamber with the sample spot toward the bottom. The sample spot is ensured to be above the level of the solvent and the chamber was closed. The plate was allowed to remain undisturbed until the solvent reaches to within 1 cm of the top. The plate was removed from the chamber and immediately the solvent was marked in front using a pencil. The distance from the spotting line (origin) to the center of each spot and from the spotting line to the solvent front was immediately measured and recorded and each component was identified.

$$R_f \text{ value} = \frac{\text{distance from origin to component spot}}{\text{distance from origin to solvent front}}$$

**Immobilization of Betalain Dye**

Calcium Chloride - 4%, Sodium Chloride -0.1N, Sodium Alginate -3.5g and Syringe were the materials required. 4% $CaCl_2$ by weight and 4 gm of $CaCl_2$ is mixed into 100 ml water. By using magnetic stirrer the solution was mixed properly. After the stirring the solution was kept at 4 degree centigrade for 2 hours. 0.1N NaCl sodium alginate solution which is prepared by adding 0.685gm of NaCl is mixed into 100 ml water. To this mixture 3.5gm of Sodium Alginate is mixed and the solution is kept for incubation. After incubation 2 - 4% of the sample is added in 10ml of 0.1N NaCl sodium alginate solution. Finally $CaCl_2$ is taken in beakers and by using a syringe Sodium Alginate solution of different colour is added drop by drop into different beakers.

**Application of Natural Dye in Food**

After the pigment was removed from the ethanol using rocker, 1 – 2 ml of betalain pigment was applied in the curd. The solution turned red in color. It was then store in the refrigerator at 20˚C.

## 3. RESULTS AND DISCUSSION

Plants extracts constitute an important source of active natural products which differ widely in terms of structures, biological properties and mechanisms of actions. Betalain showed the presence of tannins, saponins, flavonoids, reducing sugars and terpenoids (Figures 3–8; Graphs 1–4; Table 1).

### 3.1. Estimation of flavonoids in beet root (*Beta vulgaris*) extract

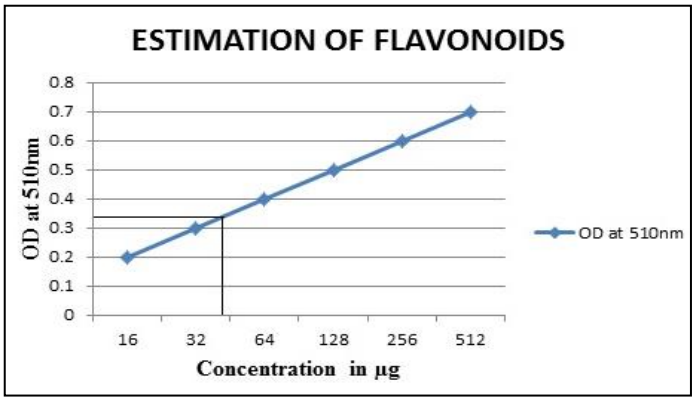
The flavonoid content in betalain was found to be 46.4 µg/ml (Graph 1).

### 3.2. Antioxidant capacity of beet root (*Beta vulgaris*) by phosphomolybdenum method

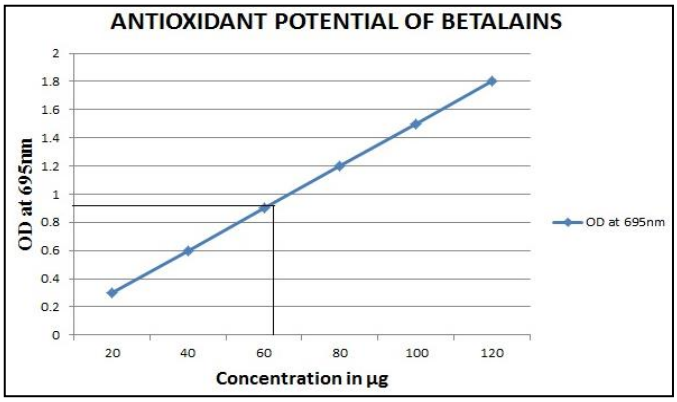
The antioxidant capacity of beet root (*Beta vulgaris*) was found to be 63µg/ml (Graph 2).

### 3.3. Stability Tests of Betalain and Anthocyanin Pigments

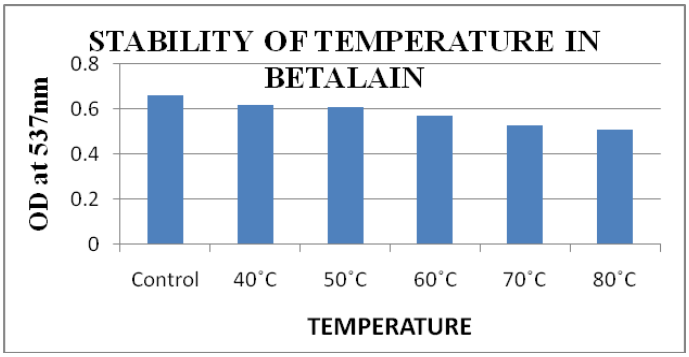
**Betalain :** It was observed that increase in temperature, decreases the color significantly. As the duration of heating increased prominent loss in color is observed .The betalain was stable only at 20˚C (Graph 3).



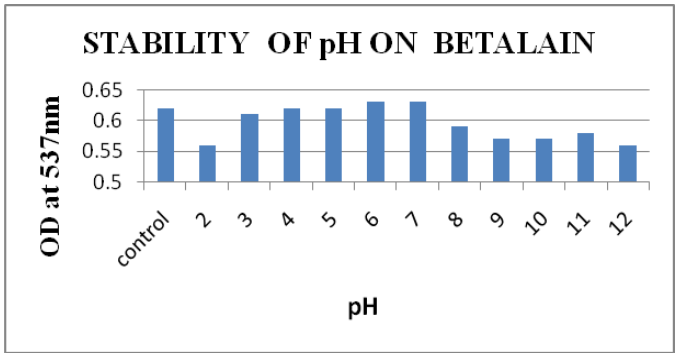

**Graph 1**
Flavonoids in Betalain




**Graph 2**
Antioxidant Potential of Betalains




**Graph 3**
Stability of temperature in betalain




**Graph 4**
Stability of ph on betalain

**Figure 9**
Column chromatography

**Figure 10**
Betacyanin

**Figure 11**
Betaxanthin

**Figure 12**
Thin layer chromatography of betacyanin

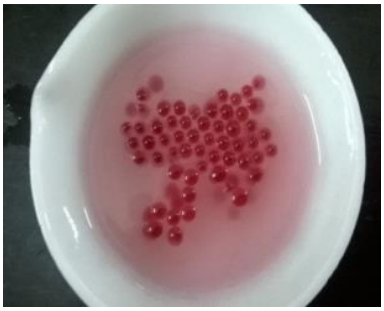


**Figure 13**
Immobilization

**Figure 14**
Betalin in curd

**Table 2**

| Pigment | Distance migrated | $R_f$ value |
|---|---|---|
| Betacyanin | 8.5cm | 0.98 |

### 3.4. Stability to PH in beet root- betalain (*Beta vulgaris*)

**Betalain:** The stable pH range of betalain was found to be 3.0- 7.0. Pigment was degraded beyond this range. So there was constant decrease in color to brown (Graph 4).

### 3.5. Purification of Betalain from beet root (*Beta vulgaris*) by column chromatography

Purification was successfully done in betalain samples of beet root. The samples gets seperated into two different compounds namely red colored betacyanin which is the major pigment present in beet root and yellow colored betaxanthin which is a minor pigment (Figures 9–11).

### 3.6. Confirmatory test of Betacyanin

**Thin layer chromatography of Betacyanin pigment from beet root (*Beta vulgaris*)**

$$R_f\text{value} = \frac{\text{distance from origin to component spot}}{\text{distance from origin to solvent front}}$$

The compound was confirmed to be betacyanin .Since the $R_f$value was found to be similar to that of the previous work done on betacyanin the $R_f$ value of betacyanin determined is 0.98 (Figure 12; Table 2).

**Immobilisation of Betalain**

When different coloured Sodium Alginate solutions is added drop by drop into different beakers containing $CaCl_2$ different colours of beads are formed. Enzymes can be safely preserved by natural dyes. Using the chemical dye for enzyme preservation technique may affect the chemical nature of the preserving enzyme which leads to some changes in enzyme activity. But if we use dye extracted from natural materials it doesn't affect the nature of enzyme. Thus Natural dye has no harmful effect on health (Figures 13).

**Application of Betalain Pigment in Food (Curd)**

The curd was coloured using betalain and anthocyanin pigments. Natural dyes can be used in various food materials because it is non-toxic and has no harmful effects. Since it is unstable to conditions such as temperature and pH it is maintained in the correct conditions (Figure 14).

## 4. CONCLUSION

Betalains are set to become increasingly important as nutraceutical ingredients (foods marketed in terms of their health benefits), as they replace synthetic food colourings. The betalains have a number of health-giving properties. Infusions of betalains from the bracts of *Bougainvillaea* mixed with honey, for example, are used to treat coughs in parts of Mexico. Some antiviral and antimicrobial activity has been attributed to betalains. This has evolved against viral and microbial pathogens. Red pigment has been shown to be active, for example, against *Pythium*, a pathogenic fungi of beets. This antiviral and antimicrobial activity could also be beneficial in medicinal terms. The main focus of interest, however, has recently been on betalain pigments as antioxidants. The presence of betalains means that beetroot has a stronger antioxidant activity than most vegetables. Antioxidants in the diet reduce the risk of cardiovascular disease and cancer. The following chapter looks at the health benefits of consuming beetroot in more detail. The stability of betalains dictates their range of food colouring applications. Despite having lower stability than synthetic food colourings, betalain pigments are widely used in food products. Betalain pigments are particularly suitable for use in food products with a short shelf-life, that have been produced with minimum heat treatment, and that are packaged in a dry state under reduced levels of light, oxygen and humidity. Natural food colourings are undergoing a revival within the food industry. Recent health scares have centred around the presence of the banned synthetic red dyes Sudan 1 and Para Red in foods. Natural pigments such as betalains may therefore become increasingly used in food products. Methods are being developed to improve the

production of betalain in beets, through plant breeding, and cell tissue culture and biotechnology. In addition to increasing the quantity and quality of betalains, the ultimate aim is to improve the stability of betalain molecules in food products. Thus the present study has proved that betalain pigment with no toxic side effects in the human body can undoubtedly be foreseen as a *natural and safe alternative to synthetic red colourings*.

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