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Extraction and Evaluation of Anthocyanin from *Dioscorea Alata* (L.) for Its Application as a Natural Food Colour

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Abstract:
Dioscorea alata (yam) is an underutilized tuber crop which is widely propagated in Asian countries. Different landraces with variation in tuber color are available in Asian and African countries. This study is focused on the extraction, characterization and application of anthocyanin pigments from purple colored yam as a natural food color. Anthocyanin content was estimated as 1.43 mg/g. Fourier transform infrared spectroscopy (FTIR) spectrum showed a single sharp transmittance peak which shows the possibility of a single anthocyanin pigment type. The pigment was thermal stable and pH stable. Applicability of the extracted anthocyanin pigment as food color was tested in ice cream, lemon juice, and hard candy. The quantity of pigment needed for coloring food item was within the permissible limit, set for food colors by U.S Food and Drug Administration (FDA).

Keywords: *anthocyanin, natural food color, Dioscorea alata, FTIR spectroscopy, purple yam*

1. Introduction

Food colorants are important components in food industry that increases the commercial value of food items. Colors make the food more attractive, visually appealing, and provide a more variable range of products, which is especially important in the confectionery industry. It is also important in the perception of flavor. Attractively colored food items stimulate the appetite more than discolored ones. Despite some controversy in this area, the coloring of food is unavoidable.

According to FDA all synthetic food colors are subjected to certification, while food colors obtained from mineral, plant, or animal sources are exempted from certification. They are not subject to batch certification requirements.

Synthetic dyes which are commonly used in food items are carcinogenic, neurotoxic, and genotoxic (Kobylewski *et al,* 2010). Other ailments caused by the artificial food colors include asthma, rhinitis, childhood hyperactivity, birth defects, still births, sterility, early foetal deaths etc. An effective alternative to the danger caused by extensive use of synthetic food colors is the discovery of new natural sources of pigments like anthocyanin, carotenoids, and chlorophyll that can be used as food colors. Anthocyanin is the major pigment that could be exploited here. It is a flavonoid and present in nature mainly in six different types (Jing, 2010).

Out of natural sources plants play an important part as they are the colorful entities in the world. Several attempts have made to isolate natural color from tissues including annatto, saffron, paprika, grape skins, caramel, beetroot, and turmeric. Another important advantage of the use of colors derived from natural sources is that many are bioactive. Anthocyanins are being valued as bioactive due to their high level of antioxidant and anti-inflammatory capacity (Miguel, 2011., Griffiths, 2005).

Some of the challenges in the utilization of natural food colorants include requirement at higher levels to impart color to food items. They unexpectedly change the texture, odor, and sometimes impart unacceptable flavor to the food. They are less stable at higher temperatures and at different pH ranges. The sources are not frequently available and the pigments are expensive. They are also more likely to be contaminated with undesirable trace metals, insecticides, herbicides, and bacteria. So an ideal natural food color must overcome these limitations. The source should be locally available and the extraction process should be cost effective.

Dioscorea alata is a species of yam belonging to family Dioscoreaceae, a tuberous root vegetable. The tuber used in the present work was bright lavender in color (purple yam).

The aim of the study was to extract anthocyanin from locally available purple yam and to evaluate its stability in terms of temperature and pH. To characterize the anthocyanin FTIR was carried out. Its applicability as a food colour was evaluated by preparing different food items using the extracted pigment. The overall acceptability was proved by hedonic scale grading.

2. Materials and Methods

The material used for the study was purple coloured *Dioscorea alata.* The purple colored tuber was collected from local market.

2.1. .Extraction of Anthocyanin Pigment from the Samples

Anthocyanin from the tuber of purple yam and was extracted by using the method of Fuleki *et al*, (1968) modified by Colin *et al*, (1980). For the extraction of 1gm tissue, 5ml ethanol (MERCK, Germany) acidified with .0005% citric acid MERCK, Germany) was used as the solvent. The outer skin was pealed and then homogenized with the extraction solvent using mortar and pestle. Refrigeration for a few minutes made the blending process easier. The extracts were centrifuged (REMI, India) at 4000 rpm for 10 min to remove the residue.

The extract was then concentrated in a rotary vacuum evaporator at 91^0C until the solvent evaporated. Quantification of anthocyanin was done for samples before and after rotary evaporation.

2.2. Quantification of Total Anthocyanin

Total anthocyanin present in the sample was quantified using the pH differential method proposed by (Wrolstad, 2005). Anthocyanin concentration was estimated by

Concentration of anthocyanin pigment (mg/litre) = $(A_{observed} \times Mw \times D_F \times 1000)/(\mathcal{E} \times 1)$

Where

Mw is the anthocyanin molecular weight (449.2 g/mol)

Ɛ is the absorption coefficient (the value available in the literature was 44900$Lmol^{-1}cm^{-1}$ for acidified ethanol)

D_F is the dilution factor

2.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR stands for Fourier Transform Infrared, the preferred method of infrared spectroscopy. The resulting spectrum represents the molecular absorption and transmission, creating a molecular finger print of the sample.

In this study, a small volume of the extract, after rotary evaporation was transferred to eppendorf tubes and were dried in a hot air oven for several days. This resulted in a hard, solid form of the pigment. A small piece of this, approximately 1µg was transferred to the sample holder in the sample compartment of the FTIR spectrophotometer and the transmittance was measured at a range of 100 nm to 1150 nm.

2.4. Stability Assays

In the present work thermal stability and pH stability assays of the extracted anthocyanin pigment from purple yam were carried out. The quantification of anthocyanin during stability assays were calculated using the method of Du and Francis, 1973

a) Thermal stability of anthocyanin pigment

In the present work a preliminary study was conducted to determine the heat tolerance of anthocyanin from purple yam for the range of temperature from 40 - 100^0 C for 30 min.

b) Effect of ph on the efficiency of anthocyanin color and its utilization as pH indicator

A preliminary study was conducted to test the stability of anthocyanin from purple yam at different pH values 2, neutral and 12 pH for 30 min. The color changes of the samples on addition of pH solutions were recorded in order to investigate the utility of the sample as pH indicator.

2.5. Application of Anthocyanin Pigments Extracted From Purple Yam

Anthocyanin from purple yam was added to food items to find out the concentration of pigment needed to color the food items. Different food items like ice cream, lemon juice and hard candy were prepared according to standard recipe.

2.6. Sensory Evaluation

Sensory evaluation of the food items were carried out using Hedonic rating scale (9 scale evaluation) (Peryam, 1952) with 12 panelists. The 9-point hedonic scale is the commonly used scale for assessing liking and preferences of foods. In this study the parameters like color, appearance, taste, and overall palatability were evaluated.

3. Results and Discussion

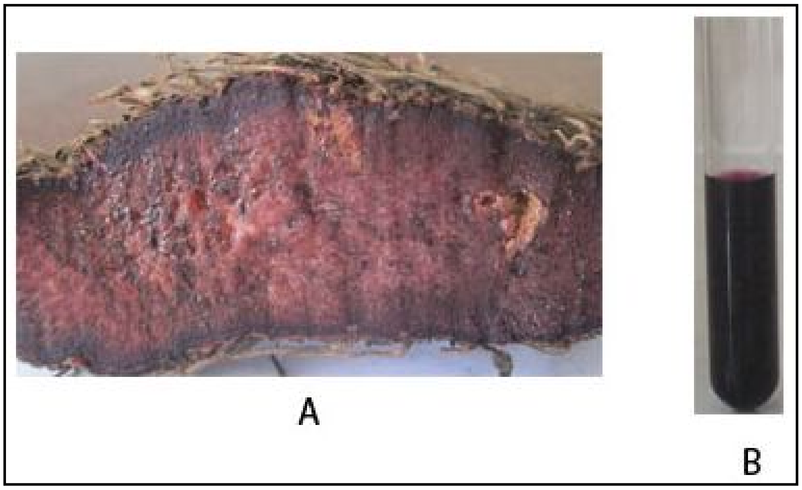

Figure 1: Dioscorea alata: A Tuber of Dioscorea alata; B Pigment extracted

3.1. Extraction and Determination of Total Anthocyanin Content Using P^h Differential Method

According to the estimation results the purple yam contains 1.43 mg/g anthocyanin. It shows that when compared to other common source like purple carrot with 1.68mg/g (Assous *et al*., 2014) anthocyanin, purple yam contains significant amount of extractable anthocyanin pigment. In addition purple yam has certain advantages over other sources as it is locally available and the extraction procedure is less expensive and simple. The average productivity of *Dioscorea* is about 30-35 tons/hectare. Pests and diseases are not common in *Dioscorea* plantation and use of fertilizers is not a prerequisite. In this respect purple yam have potential to act as an effective anthocyanin source.

3.2. FTIR Spectroscopy

FTIR spectroscopy confirmed the of presence of anthocyanin in *Dioscorea* extract.

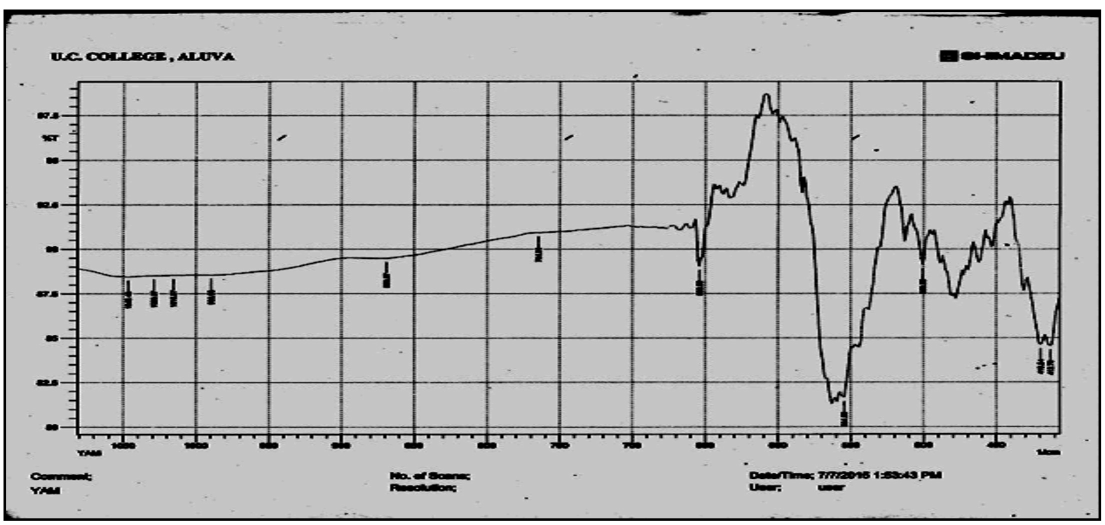

Figure 2: FTIR spectrum – anthocyanin from Dioscorea (400nm – 1050nm)

From the FTIR spectrum shown in fig. 2, it can be observed that there was a single sharp transmittance peak and a few minor transmittance peaks. The sharp peak was at 554 nm wavelength, and according to previous studies it can be understood that it represented the anthocyanin type Cyanidin – 3 – O - rutinoside. Cyanidin is the major type of anthocyanin found in fruits. The important minor peaks were observed at various nanometers like 412 nm, 419 nm, 500 nm and 653 nm. This showed the presence of other types of anthocyanin like pelargonidin -3-O- monoglucoside (500nm), cyanidin - 3- O- glucoside (419nm) etc. and other type of pigments that contribute to the property of the plant tissue.

3.3. Thermal Stability

Thermal stability of anthocyanin pigment is an important aspect that ensures its applicability as a food color. The results are shown in fig. 3

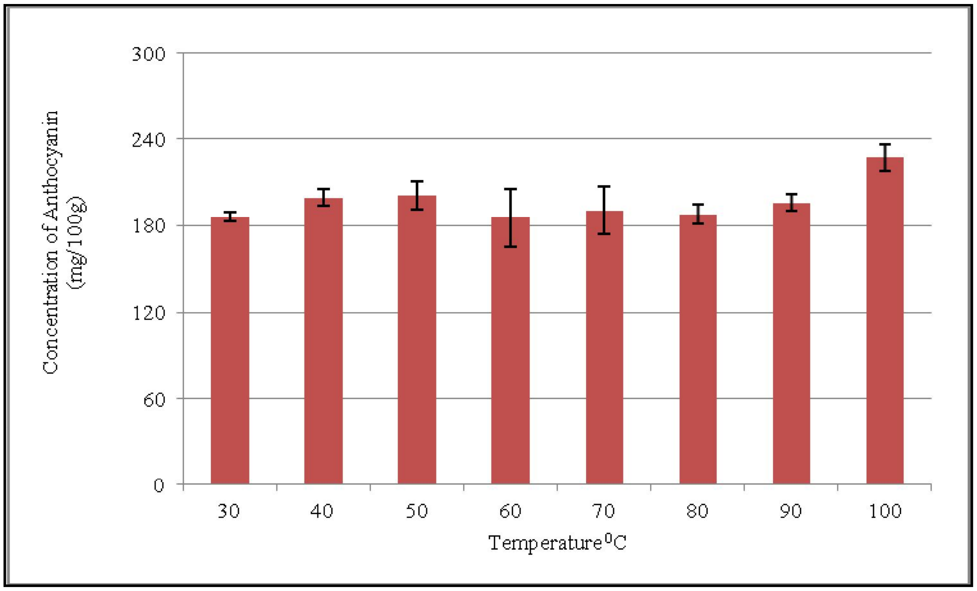

Figure 3: Thermal stability of Dioscorea pigment

The concentration of anthocyanin before the thermal treatment was recorded at 30^0C. There was a minute concentration difference after the different temperature treatments. Analysis of the above observations showed that, there was not much degradation of anthocyanin in these temperature ranges.

For food processing applications different range of temperature is needed. An ideal food colour should remain without fading. From temperature stability assay it was found out that anthocyanin from purple yam was thermal stable over a wide range of temperature and has potential to use as a food color.

3.4. pH stability

Another parameter which is important for an ideal food color is its pH stability. The results obtained from pH stability assay were represented in fig. 4

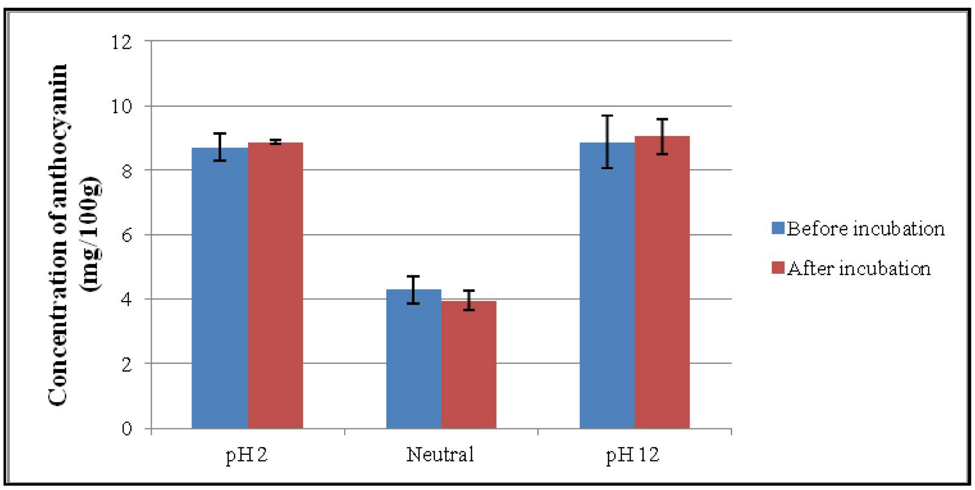

Figure 4: pH stability of Dioscorea pigment

From fig.4 it was found out that the anthocyanin content in *Dioscorea* showed relatively high stability at the pH values studied. A reduction in the absorbance in at neutral pH is due to the structural modification in anthocyanin due to oxidation. This proved the possibility of anthocyanin pigment from *Dioscorea* for coloring food items with in different pH ranges.

3.5. Application as Food Color

The anthocyanin pigment extracted from *Dioscorea* was used in various food items like ice cream, lemon juice and hard candy as colorant. The food items colored using the natural food color were then used to carry out sensory evaluation, and the ratings were recorded at the 9 point - hedonic scale. The ratings were analysed for checking the applicability of the pigment as natural food color.

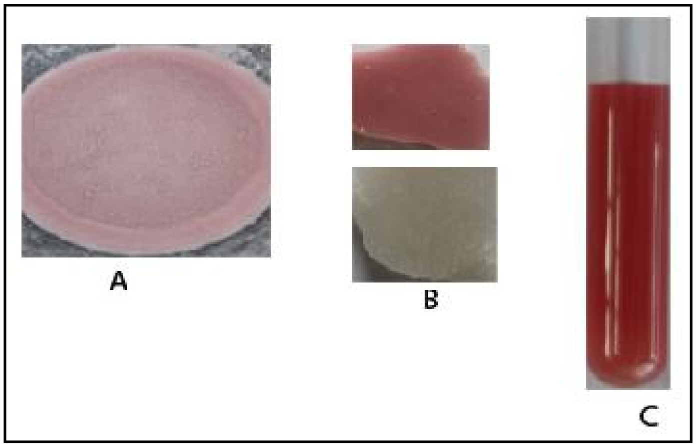

Figure 5: Images of food items prepared using anthocyanin pigment from Dioscorea: A Ice cream; B Hard candy: colored using anthocyanin (pink) and control without food color (white); C Lemon juice

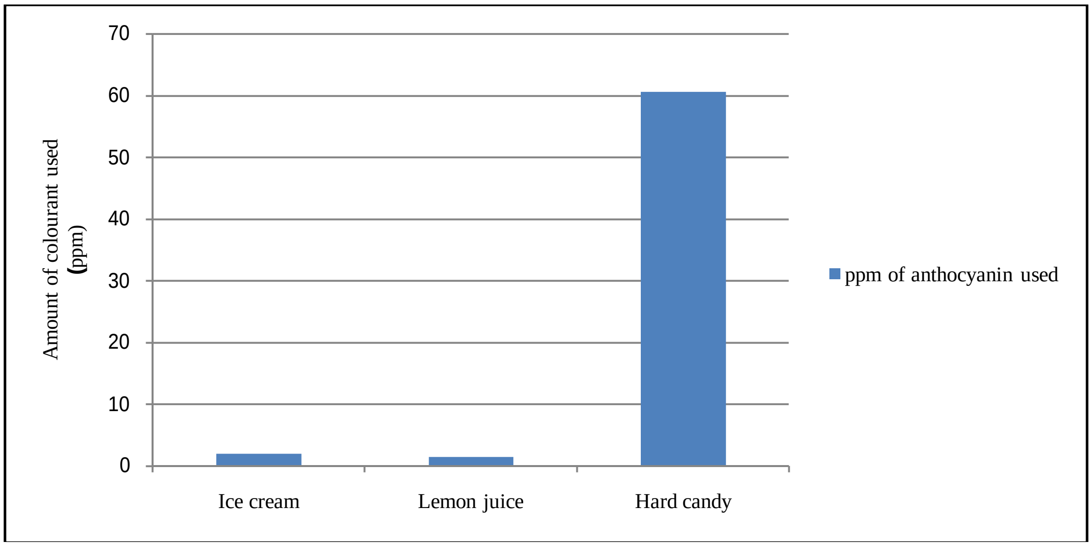

Figure 6: Amount of anthocyanin pigment used to color prepared food items

a) Ice cream
It can be inferred that relatively a little amount of pigment about 20.24 ppm was required to impart color to the ice cream. The result of requirement of anthocyanin pigment from *Dioscorea* for ice cream is shown in fig.6. The requirement of anthocyanin from *Dioscorea* is lesser than the permitted content of natural food color by FDA. .
During the sensory evaluation, it was found out that the ice cream colored with anthocyanin from purple yam showed acceptable ratings (above 7.5 in a 9 point hedonic scale) from the hedonic scale. The results are shown in table 1.

Mean: mean of the ratings recorded in the 9 point hedonic scale for the 4 parameters

	Ice Cream		Lemon Juice		Hard Candy	
Hedonic Scale Parameters	**Mean Rating**	**SD**	**Mean Rating**	**SD**	**Mean Rating**	**SD**
Colour	8.08	± 0.75	8.00	±0.70	8.25	±0.92
Appearance	7.83	± 0.37	8.00	±0.91	8.16	±0.89
Taste	8.25	± 0.72	8.33	±0.74	7.66	±1.02
Overall Palatability	8.00	± 0.57	8.00	±0.57	7.91	±0.86

Table 1: Mean of ratings and standard deviation of the four parameters of hedonic scale of food items prepared

b) Lemon juice
Lemon juice is an acidic food item. When the food colors were added it gave a red-orange color to the food item. About 1.61 ppm pigment was only needed to color the previous formulation of lemon juice. The amount of anthocyanin required was within the

acceptable range of concentration permitted by FDA. Hence the applicability of the sample under study was confirmed in coloring soft drinks.
The use of anthocyanin from purple yam as a natural food color was proved by the ratings shown in table 1.

c) Hard candy
Similar to other food items tested it can be observed that relatively lesser amount of the pigment about 60.72 ppm was needed for coloring hard candy. The result is shown in fig. 5. The quantity of pigment needed to color hard candy comes under the acceptable range of food color concentration. Hence the applicability of the pigment from the sample under study as food color was confirmed.
Hedonic scale analysis proved that the ice cream colored with anthocyanin from purple yam showed acceptable ratings in the hedonic scale.

3.6. pH Indicator
The result was similar with the results of Sushma Bondre., 2012 in *Rubus occidentalis. Dioscorea* showed a red color at pH 2, a faint red at neutral pH and a green color at pH 12 as shown in fig.7. So the results supported the observation that anthocyanin could be used as a pH indicator, and confirmed the utilization of *Dioscorea* as a pH indicator.

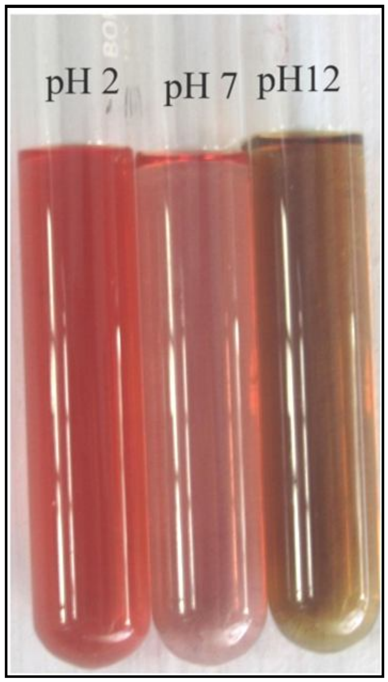

Figure 7: Utility of anthocyanin from Dioscorea as pH indicator

4. Conclusion
Anthocyanin can be isolated by simple extraction procedures from *Dioscorea alata* . The extracted pigment was temperature stable and pH stable. Pigment was characterized by FTIR spectroscopy. The pigment extracted could be effectively used as food colour within the permitted range of quantity stipulated by FDA. Sensory evaluation proved the utilization of *Dioscorea* pigment as a natural food color.

5. Acknowledgment
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