The Pharma Innovation Journal 2019; 8(1): 42-47

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(1): 42-47

www.thepharmajournal.com
Received: 26-11-2018
Accepted: 30-12-2018

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Development and characterization of biocolor (*Brassica oleracea var. capitata* F. *rubra*) fortified lime squash

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Abstract
The major pigment of purple cabbage is anthocyanin, responsible for many of the attractive colors, from scarlet to blue. It represents about 196.5 mg of polyphenols per 100g of raw purple cabbage that can be determined spectrophotometrically. Purple cabbage also contains high amount of dietary fiber, carbohydrates and related bioactive compounds. It is thus significant and even essential to find applications in different food products (e.g. Lime Squash). Squash is a non-alcoholic concentrated syrup used in beverage making. It is usually fruit-flavored, made from fruit juice, water, and sugar or a sugar substitute. In this study color extracted from purple cabbage was used for the development of lime squash, packed in glass bottles and sealed hermetically and non-hermetically using wax. The polyphenol of all samples were evaluated spectrophotometrically and expressed in mg Gallic acid equivalent/gm of sample. The light absorbance and color stability of the sample were studied using UV-Vis double-beam spectrophotometer. In this study, the peak of anthocyanin is processed in between 500-600 nm and the maximum peak height of anthocyanin is found at 560nm and color stability is determined within the wavelength range between 550-560nm. The best result in all aspects was found for the lime squash sealed hermetically.

Keywords: purple cabbage, anthocyanin, lime squash, gallic acid equivalent, color stability

1. Introduction
Color is a measure of quality and nutrient content of foods. The objective of adding color to foods is to make them appealing, augment the loss of color during processing, to improve the quality and also to influence the consumer to buy a product. At present, the demand for natural dyes is increasing worldwide due to the increased awareness on therapeutic and medicinal properties and their benefits among public and also because of the recognized profound toxicity of synthetic colors. Natural dyes are those derived from naturally occurring sources such as plants, insects, animals and minerals. Among all the natural dyes, plant-based pigments have medicinal values so are mostly preferred (Chaitanya, 2014). The major pigments present abundantly in purple cabbage are anthocyanin which can be used as a source of natural color. The term anthocyanin was derived from the Greek *anthos*, a flower, and *kyanos*, dark blue. Anthocyanins are the most important group of pigments, after chlorophyll that is visible to the human eye (Harborne *et al*., 1988). Anthocyanins are responsible for many of the attractive colors, from scarlet to blue, of flowers, fruits, leaves, and storage organs (Harborne *et al*., 1988-1993). Being part of flavonoids, anthocyanins are the greatest natural pigments giving blue color in plants and showing antioxidant potential.
Purple cabbage belonging to *Brassicaceae* family is a vegetable with high total antioxidant capacity being also very rich in minerals, vitamins, polyphenols, anthocyanins and glucosinolates. It represents about 196.5 mg of polyphenols per 100g of raw purple cabbage. Purple cabbage also contains high amount of dietary fiber, carbohydrates and related bioactive compounds. Purple cabbage, is more unique among the cruciferous vegetables in providing a big quantity of anthocyanins, which qualify not only as antioxidant nutrients, but also as anti-inflammatory nutrients. The antioxidant richness of cabbage is partly responsible for its cancer prevention benefits (Draghici *et al*., 2013) [5].

2. Materials and Methods
2.1 Materials
2.1.1 Raw Materials Required: Raw materials for the preparation of this squash such as purple cabbage, sugar, limes were brought from local market adjacent to the institution.

The class II preservative (KMS), citric acid and bottles for packaging were available in the departmental laboratory.

2.2. Methods

2.2.1 Extraction of color of purple cabbage: Purple Cabbage was brought from local market (Dumdum, Kolkata). It was washed and chopped finely. Citric acid solution was added with chopped purple cabbage in 2:1 ratio. The mixture is then cooked at low flame for 15minutes. The extracted bio-color is strained by using strainer. The volume of extracted bio-color was measured for preparing yield of the color.

2.2.2 Preparation Color fortified Lime Squash: The limes were washed thoroughly and peeled. They were cut into small pieces (seeds are discarded) for easier extraction of juice. The juice was extracted from the fruit by using pulper. After that the juice was strained with muslin cloth to remove the pulp if any present in the juice. The total solid content of fruit juice mainly sugar was measured by using refractometer and the acidity and vitamin C content was measured. According to quantity, the 40% sugar and 30% water needed were measured for squash preparation. Simultaneously, the color extract was also prepared from purple cabbage by acid extraction method. The cabbage was washed thoroughly and weighed. It was cut and grounded with 200ml 0.1(N) citric acid. It was then filtered through muslin cloth to get clear color extract. After that, sugar syrup was prepared and allowed to cool down. Simultaneously, glass bottles were sterilized in hot water bath and then they were kept inside tray dryer at 63.5°C for complete drying. After cooling, the sugar syrup was mixed with the fruit juice thoroughly and class II preservative was added to the squash. Finally, the product was poured into hot sterilized bottles and sealed with plastic caps. One bottle was sealed hermetically using hot wax to check color stability during storage. Further they were stored at low temperature (7°C).

2.2.2.1 Composition of Ingredients

Table 1: Composition of Ingredients

Sample Volume	Sugar	Water	Citric Acid	KMS
Purple colored lime Squash (950ml)	440.1grams	416.4ml	13.47gram	406mg

2.2.2.2. Flow chart for preparation of purple cabbage color fortified lime squash

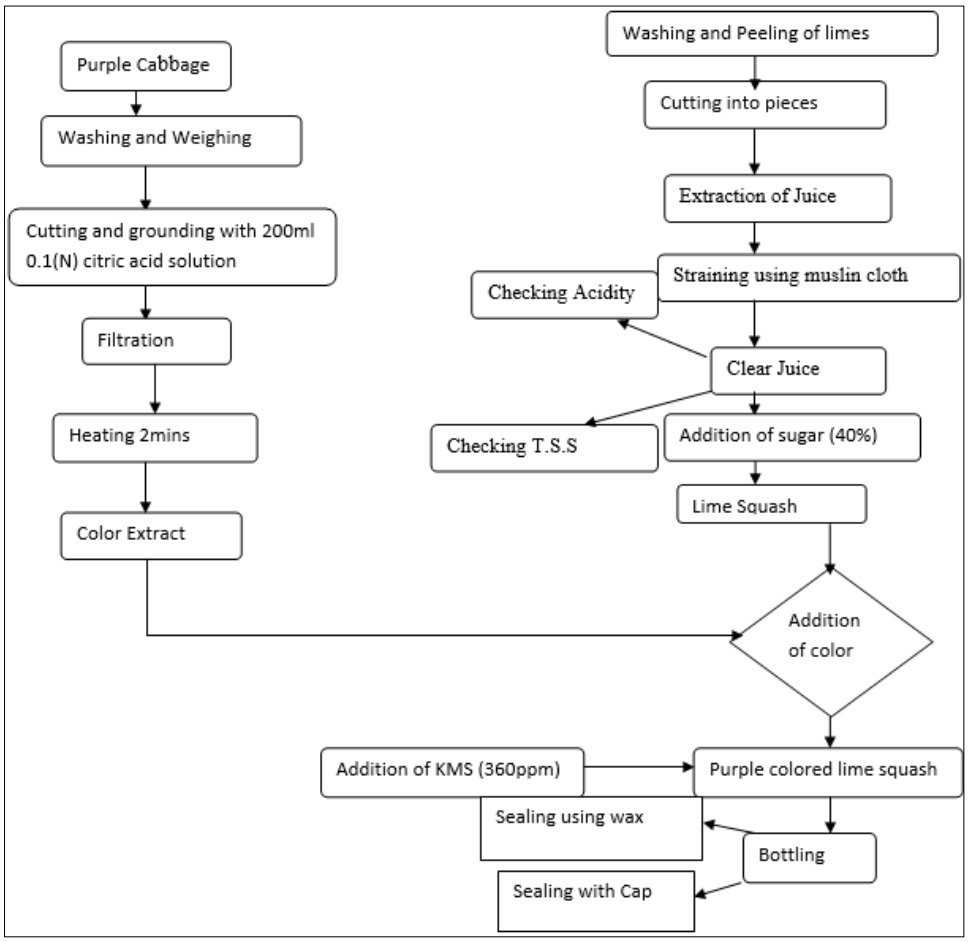

Fig 1: Process Flow chart of Purple cabbage color fortified lime squash

2.2.3. Quality Analysis of the Squash sample

A. Physical Analysis

2.2.3.1. Determination of total soluble solids: The total soluble solids of squash is calculated using standard method of I.S., 1993(Ref:-I.S 13815:1993/I.S.O. 2173:1978 Fruits and Vegetable products Determination of Soluble solids Refractometer method).

2.2.3.2. Estimation of Color Stability of Squash Sample: The absorbance of sample both sealed and unsealed is

measured by using UV-Vis double-beam sprectophotometer (Jasco V 630 Sprectphotometer) to determine the color stability.

B. Chemical Analysis

2.2.3.3 Determination of Acidity: The acidity of squash is calculated using standard method of A.O.A.C, 2000. (Ref: - AOAC 17th edition, 2000, Official method 942.15 Acidity (Titrable) of fruit products read with A.O.A.C official method 920. 149 Preparation of test sample).

2.2.3.4 Determination of polyphenol content of extracted anthocyanin by using solvent: Methanol as a solvent was used for polyphenol content extraction. The polyphenol content was determined by using UV-Vis double-beam sprectophotometer (Jasco V 630 Sprectphotometer) by standard Mcdonald *et al*. (2001) method and expressed in terms of Gallic acid equivalent (GAE) (Merzlyk *et al.*, 2013).

2.2.3.5 Determination of the stability of color Extract

Stability extracted color in different pH was determined by mixing different volume of 0.1 (N) HCl and 0.1 (N) NaOH to the extracted colored solution. pH was determined by using digital pH meter (pH meter CL 46+, Toshcon Industries Pvt., Ltd.). The absorbance of the color extract in different pH solution (by addition of acid and alkali) were determined by using UV-Vis spectrophotometer (Jasco V 630 Spectrophotometer) at 560nm wavelength.

C. Sensory Evaluation

Sensory characteristic of the product was analyzed. It was mainly done with the help of 9-point hedonic scale (Hooda and Jood, 2005). The scale was as follows:

Table 2: 9 point Hedonic Scale

Range	Point
Like Extremely	**9**
Like Very Much	8
Like Moderately	7
Like Slightly	6
Neither Like Not Dislike	5
Dislike Slightly	4
Dislike Moderate	3
Dislike Very Much	2
Dislike Extremely	1

3. Results and Discussions

3.1 Results

3.1.1 Physiochemical analysis of Squash

Table 3: Physiochemical analysis of Squash

S. No.	Sample	Acidity	T.S.S	% Juice	Sealing
1.	Sample1	1.4%	45°brix	8%	cap
2.	Sample 2	1.5%	42°brix	15%	Sealing with wax
3.	Sample 3	1.56%	41°brix	15%	Cap

3.1.2. Estimation of polyphenol content: The polyphenol content of the samples were estimated using spectrophotometer at 560nm. The polyphenol content of sample1, sample2 and sample3 were 215mg GAE/gm, 305.6mg GAE/gm and 230mg GAE/gm.

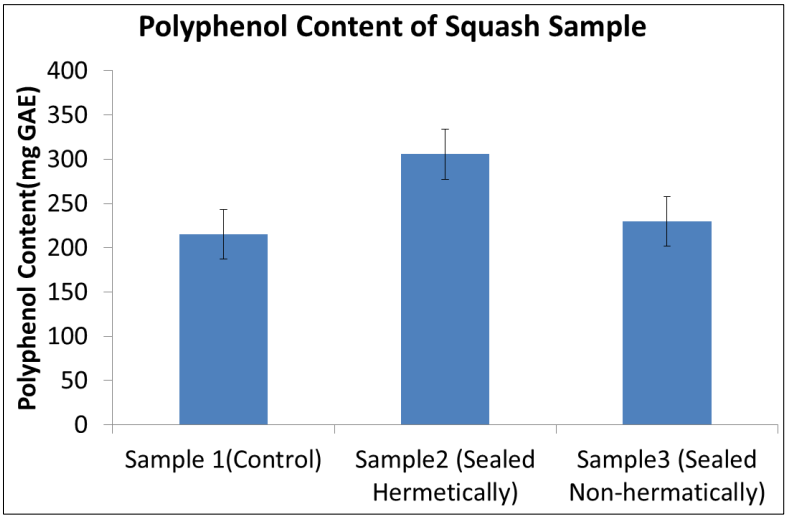

Fig 2: Polyphenol Content of Squash Sample

3.1.3. Determination of Peak height of Anthocyanin: The peak of anthocyanin is processed spectrophotometrically in between 500-600 nm and the maximum peak height of anthocyanin is found at 560nm.

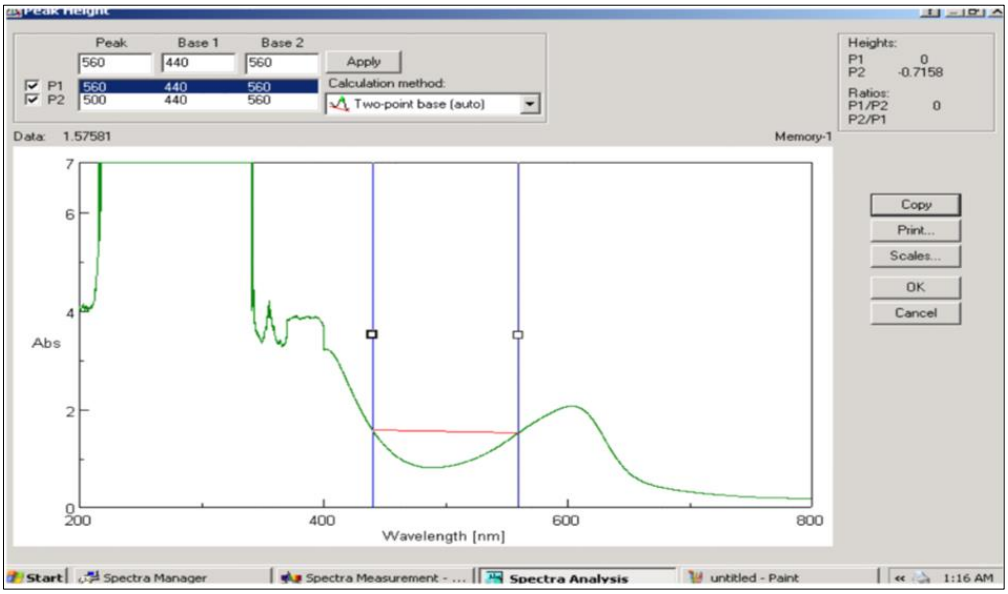

Fig 3: Peak height of anthocyanin

3.1.3. Stability of Anthocyanin at Different pH: The color has been extracted at pH 1.5-1.56. pH of extracted color was adjusted by addition of acid and alkali and it ranges between 1.5-9.5 with changing in color from Purplish red to olive green.

3.1.4. Estimation of Polyphenol Content of Color Extract: The polyphenol content of color extract different pH was estimated in terms of GAE/gm of sample at 765nm. The highest polyphenol content was 130.85 mg GAE/gm at pH 5.5.

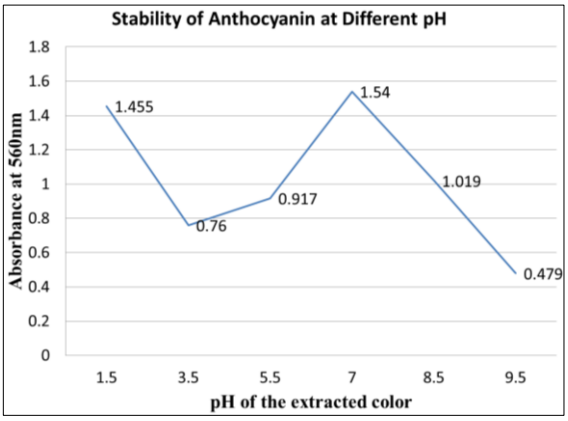

Fig 4: Stability of Anthocyanin at Different pH

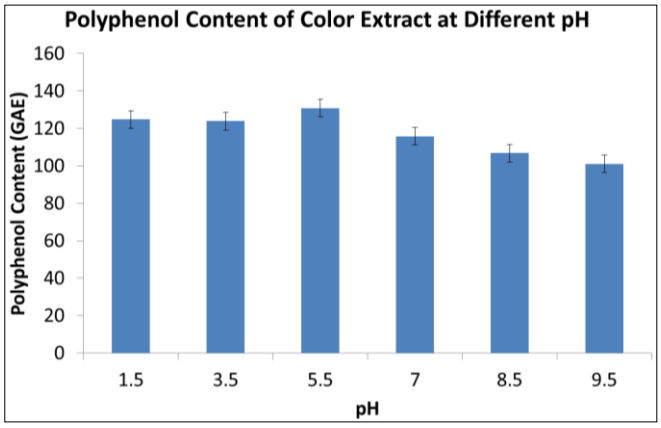

Fig 5: Polyphenol Content of Color Extract at Different pH

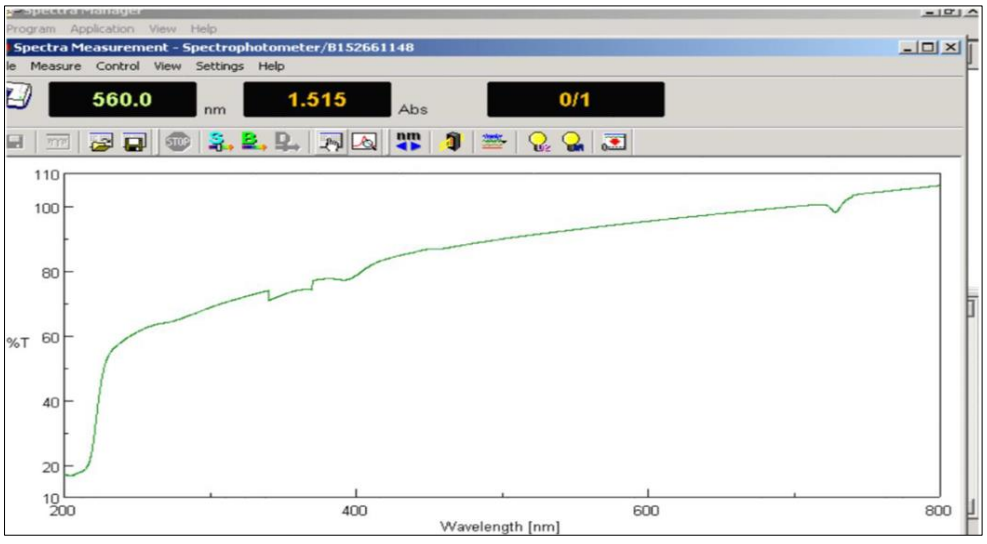

Fig 6: Absorbance of sealed sample

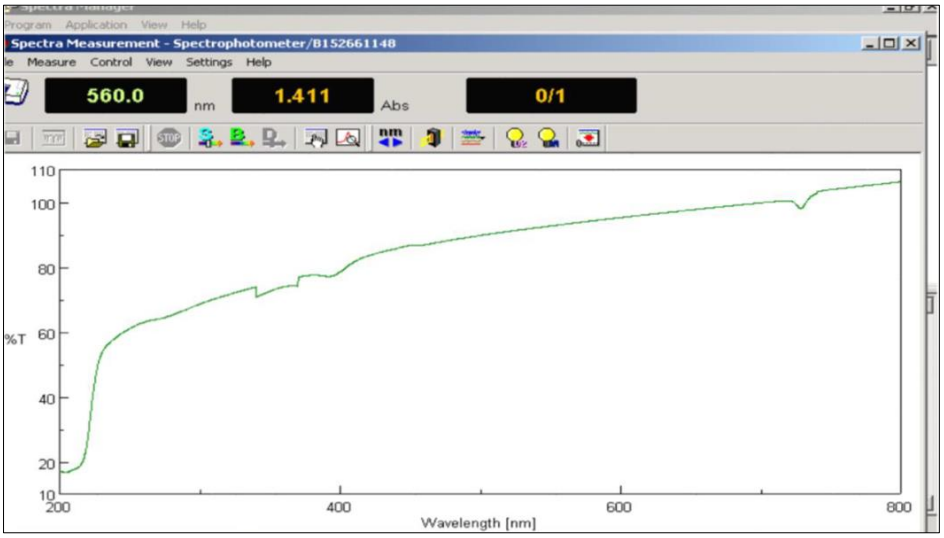

Fig 7: Absorbance of unsealed sample

3.1.5 Storage studies of fortified lime squash: Storage stability of fortified lime squash has been studied at 7days interval. During this period, the samples were stored under refrigerated condition at a temperature of 7°C. The staility of color was checked spectrophotometrically at 560nm. Maximum color stability was observed for hermetically sealed sample.

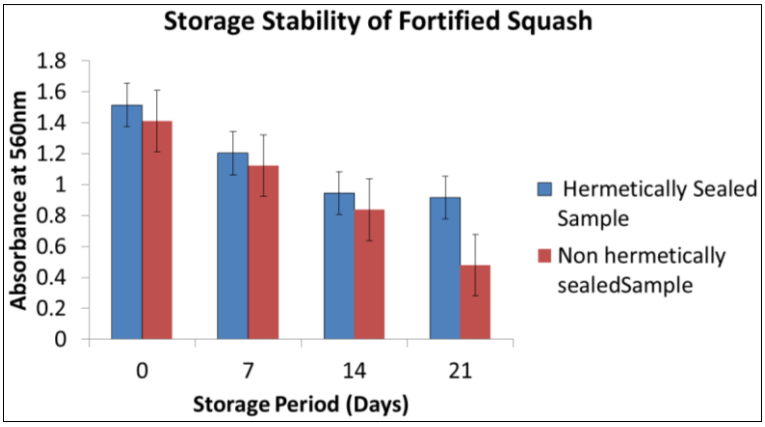

Fig 8: Storage Stability of Fortified Squash

3.1.6 Sensory evaluation of fortified lime squash
Sensory analysis of squash samples is evaluated in 9 point hedonic scale. It is found that in case of the purple cabbage color fortified lime squash sealed hermetically, the color and flavor are about 9.0, body texture and mouth feelness are about 8 and overall acceptance is about 8.5 more than the control and sealed non hermetically sealed samples.

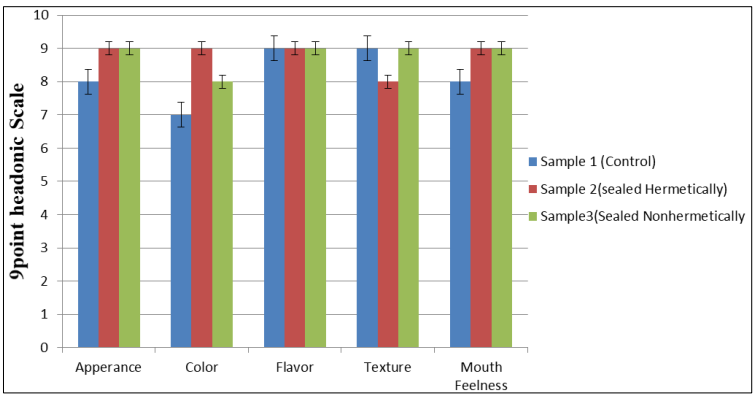

Fig 9: Sensory Evaluation of Fortified Lime Squash

4. Conclusion
Regular intake of rare vegetable rich diet or their extract fortified rich diet plays an important role in human nutrition. They have undeniable positive effects on health since phytonutriceuticals of rare vegetables can protect the human body from several types of chronic diseases. In this project,

many trials have been done with different modifying processes. As I have already discussed about sensory evaluation of both of the product in the first trial the product became much sweeter and appearance is not so appealing so the process is modified and problem has been overcome. The improper sealing has an adverse effect on purple cabbage color fortified lime squash. Among three samples of on purple cabbage color fortified lime squash, sample 2 i.e. sealed with wax gives the satisfactory result because it attributes all characteristics i.e. sensory, nutritional and physiochemical evaluations are optimum.

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