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# ANALYSIS OF CHLOROPHYLLS, CAROTENOIDS AND ANTHOCYANIN CONTENT DURING RIPENING OF GREEN CHILIES

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Chili peppers are one of the widely used popular species with multitude of health benefits. Apart from the capsaicin, chilies also contain various pigments which impart many beneficial effects on human health. The concentration of different pigments like chlorophyll, carotenoids, anthocyanin varies according to the ripening stage. The present study was conducted to evaluate the change in the pigment content in different stages. It was found that the concentration of chlorophyll decreases and the level of anthocyanin and carotenoids increase during storage and ripening of chilies. The observation of the study reveals that the concentration of chlorophyll-b was found to be greater than chlorophyll-aduring all stages of estimation. The chlorophyll content predominates in the initial stage. There is a decrease in the chlorophyll content and a simultaneous increase in the concentration of both carotenoids and anthocyanin during the last stage of study period.

***Keywords:*** Chlorophyll, Anthocyanin, Carotenoids, Antioxidant

## INTRODUCTION

Chilies peppers (*Capsicum annuum* L.) are one of very popular spices used in the Indian cuisine by all sections of the society. Chilies are popular because of their medicinal as well as other health benefitting properties. Chilies are also used in various sauces, instant noodles etc. Chilies contain an alkaloid namely, capsaicin, has been shown to have many medicinal property like anti-diabetic, anti-carcinogenic and analgesic property. Chilies are excellent source of many vitamins like A, B and C. Chilies also acts as gastrointestinal de-toxicants. Chili is also known to stimulate the release of endorphins which act as natural painkillers. Chilies are known to be good source of minerals like potassium, manganese, iron and magnesium.

Chlorophyll, the major pigment present in chili has multitude of benefits to human health. Chlorophyll is considered as a super-food because it is rich in vitamin A, vitamin C, vitamin E, vitamin K and beta carotene. Chlorophyll is recognized to be a strong antioxidant. It is also rich in important minerals like iron, magnesium, calcium and potassium. Chlorophyll helps in oxygenating and cleansing the blood. Researchers have demonstrated that chlorophyll to be effective against cancer. Multiple studies have shown that chlorophyll helps to neutralize toxins in our bodies. It also helps to maintain healthy and youthful skin. It helps in digestion and also helps in maintaining the intestinal flora. Chlorophyll is also an effective deodorizer which helps to reduce bad breath and body odor. It has been shown by researchers to have anti-carcinogenic and anti-mutagenic properties. Chlorophyllin, a semi-synthetic derivative of chlorophyll have protective effect against oxidative damage (Zhang *et al.*, 2012).

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Carotenoids are a group of pigment present in many fruits and vegetables which are responsible for yellow to red color. Many carotenoids have been identified of which around 100 play an important role in our diet (Kaulmann *et al.*, 2014). Carotenoids are known as important dietary source of vitamin A and it is also known for its antioxidant property (Paiva and Russell, 1999). The beneficial property of carotenoids is due its antioxidant property. Carotenoids are known as potent destroyers of free radicals. This is of particular importance to human health because an increase in the reactive oxygen species is the major factor for various chronic disorders like cancer, cardiovascular diseases and diabetes.

Plant pigment anthocyanin is water soluble which imparts the blue, purple and red color to the plant tissues. It has many known positive health effects. It has been shown to be rich in antioxidants. The antioxidant property of anthocyanin has been shown to protect pancreatic β-cells from glucose-induced oxidative stress (Al-Awwadi *et al.*, 2005). Research by Jayaprakasam and his co-workers have demonstrated that the release of insulin from pancreatic cells is induced by anthocyanin. A decrease in triglycerides and increase in HDL-cholesterol levels were seen in experimental animals when given an extract of anthocyanin. (Al-Awwadi *et al.*, 2005). Research by Bazzanoc *et al.* (2002) has been shown that intake of anthocyanin is associated with low prevalence of disease like cancer and cardiovascular diseases. Anthocyanin consumption has been associated with lower risk of cardiovascular disease and improvement of visual function (Ghosh and Konishi, 2007). There are evidences of cancer chemoprevention by anthocyanin (Hou DX, 2003). Owing to the bright color, anthocyanin is also used as natural colorants in the food industry. Due to the understanding and awareness of the potential beneficial effects of chlorophyll, carotenoids and anthocyanin present in chilies on human health, the present study was designed to quantify the amount of the various pigments in chilies at different stages of ripening.

## MATERIAL AND METHODS

Green chilies were procured from the local market. Healthy and uninfected samples were washed thoroughly with sufficient water and used for the analysis for the determination of chlorophylls (chlorophyll-a, chlorophyll-b and total chlorophyll), carotenoids and anthocyanin. Chilies at different stages of ripening were used for the estimation of the pigment. All the solvent namely acetone, methanol and hydrochloric acid used for the extraction were reagent grade. To maintain the accuracy of the result the samples were taken in triplicates. The extraction and estimation of various pigments were done in 5 stages (Stage 1- Day 1 of purchase, Stage 2 – Day 5 of purchase, Stage 3 – Day 10 of purchase, Stage 4 – Day 15 of purchase, Stage 5 – Day 20 of purchase)

### Estimation of Chlorophylls

Accurately weighed 0.5 g of chilies which were in different stages of ripening. The samples were ground in a mortar and pestle with 10 ml of 80% acetone. The ground samples were subjected to centrifugation at 5,000 rpm for 15 minutes. The supernatant was collected in a fresh flask and the pellet was extracted again with 5 ml of 80% acetone. This procedure was continued until the pellet becomes colorless. All the supernatants were pooled and the absorbance was measured at 645 nm and 663 nm in a UV-visible spectrophotometer for the estimation of chlorophyll a and chlorophyll b.

Chlorophyll mg/g fresh weight can be calculated by the formula given below:

$$\text{Chl a (mg/g)} = [\{(12.7 \times ABS663) - (2.69 \times ABS645)\} \times V]/1000 \times W$$

$$\text{Chl b (mg/g)} = [\{(22.9 \times ABS645) - (4.68 \times ABS663)\} \times V]/1000 \times W$$

V = Volume of acetone used

W = Weight of sample taken

### Estimation of Carotenoids

Chilies in different stages of ripening were taken. Weighed 0.5 g of the sample and ground in a mortar and pestle with a solvent mixture of Petroleum ether: acetone (1:1). The ground samples were centrifuged and the supernatant saved for the estimation of carotenoids. The concentration of carotenoids was estimated by the method of Lichtenthaler and Wellburn (1983).

### Estimation of Anthocyanin

The extraction liquid for the estimation of Anthocyanin is Methanol: HCl : Water (90:1:1). The methanolic extract of anthocyanin was used for the estimation. Spectrophotometric analysis of anthocyanin pigment was carried out in a UV-visible spectrophotometer with a 1cm light path. The anthocyanin content was estimated by following the method of Murray and Hackett (1991) as $A_{530} - A_{653}$.

## RESULTS AND DISCUSSION

The changes in the chlorophyll content of chilies are tabulated in Table 1. A steady decrease in all the forms of chlorophyll was noted during the ripening of chilies. Figure 1 represents the content of chlorophyll-a during the different stages of study. The concentration of chlorophyll-b represented in Figure 2 was found to be greater than chlorophyll-a during all stages of estimation.The total chlorophyll concentration varied from 0.377 mg/g in the first stage to 0.002 mg/g in the fourth stage which gradually recorded zero in the last stage. Figure 3 shows the concentration of total chlorophyll during the study period. The concentration of carotenoids and anthocyanin during of the course of study has been tabulated in Table 2. The concentration of carotenoids was 0.011 mg/g in the initial stage but it increased to 0.238 mg/g in the third stageas shown in Figure 4. After the third stage, the concentration of carotenoids decreased and was found to be 0.135 mg/g in the final stage. The final stage was dominated by anthocyanin pigment.In the first stage the concentration of anthocyanin was measured to be 0.003 mg/g depicted in Figure 5. Anthocyanin concentration increased steadily and dominated the final stage with a concentration of 0.227 mg/

**Table 1: Concentration of Chlorophyll During Different Stages of Study**

| | Chlorophyll-a (mg/g) | Chlorophyll-b (mg/g) | Total Chlorophyll (mg/g) |
|---|---|---|---|
| Stage 1 | 0.156 | 0.221 | 0.377 |
| Stage 2 | 0.06 | 0.14 | 0.2 |
| Stage 3 | 0.056 | 0.085 | 0.14 |
| Stage 4 | 0.002 | 0 | 0.002 |
| Stage 5 | 0 | 0 | 0 |

**Table 2: Concentration of Carotenoids and Anthocyan in During Different Stages of Study**

| | Carotenoids (mg/g) | Anthocyanin (mg/g) |
|---|---|---|
| Stage 1 | 0.011 | 0.003 |
| Stage 2 | 0.113 | 0.12 |
| Stage 3 | 0.238 | 0.029 |
| Stage 4 | 0.213 | 0.123 |
| Stage 5 | 0.135 | 0.227 |

**Figure 1: Chlorophyll-a Content During the Study Period**

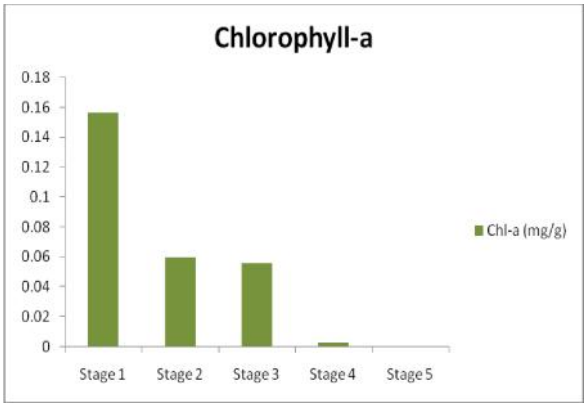



**Figure 2: Chlorophyll-b Content During the Study Period**

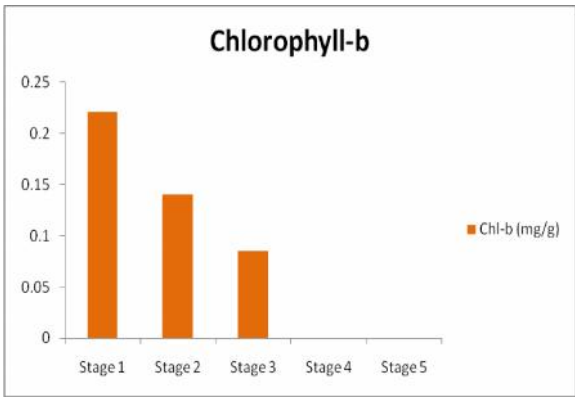



**Figure 3: Total Chlorophyll Content During Various Stages of Study**

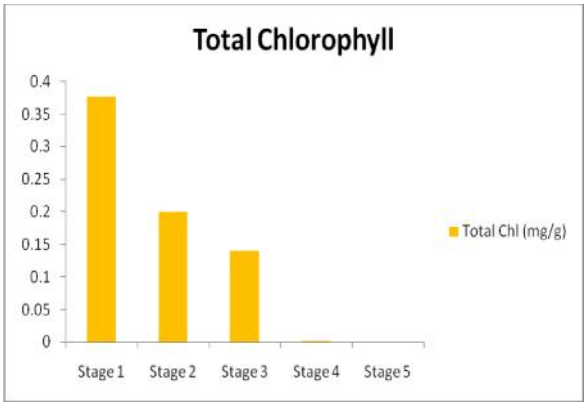


**Figure 4: Carotenoids Content During the Study Period**

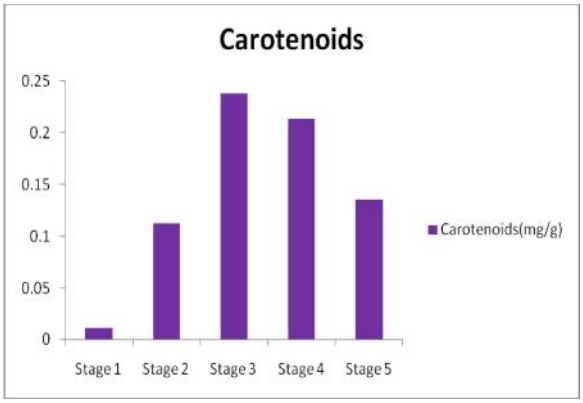



**Figure 5: Anthocyanin Content During the Study Period**

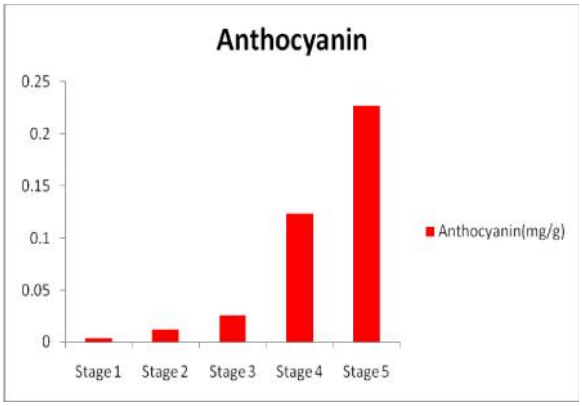
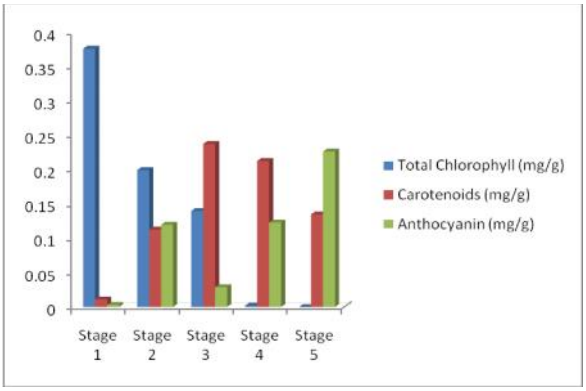



**Figure 6: The Graph Showing the Comparative Concentration of Total Chlorophyll, Carotenoids and Anthocyanin**




g. Figure 6 shows a comparative picture of the concentration of all the pigments during the study period.

The result from this study will add to the already existing data on the usefulness of chilies. Chili is extremely popular and has many useful properties. The pigments present in the chili are one of the main reasons for the beneficial effect of chili. The pigments chlorophyll and carotenoids are the principal pigments responsible for the green to yellow color of the vegetables and fruits (Knee, 1972). The quality of the fruits and vegetables is determined by the content of pigments present in it and the changes involved during its ripening. The proportion of different pigments like chlorophyll, carotenoids and anthocyanin determine not only the attractiveness but also the quality of chilies. The final stage of estimation shows a high concentration of anthocyanin of about 0.227 mg/g maintaining the attractiveness of chilies.

The chlorophyll content is an indicator of ripeness. The chlorophyll, carotenoids and the anthocyanin content are of great importance because of its antioxidant properties and other nutraceutical effects it possess (Tournaire, 1993: and Rice Evans 1997). The ripening of chilies is associated with increase in anthocyanin and carotenoid contentand decline in the concentration of both chlorophyll a and b.

During ripening, the pigment chlorophyll is unstable and is replaced by other pigments. There is a continuous breakdown of chlorophyll pigment which enables the pigments carotenoids and anthocyanin to show through. Carotenoids are a group of phytochemicals which are known for its potent antioxidant property. Merzylak and Chivkunova (2000) suggest the protective effect of anthocyanin against harmful UV radiation. Consumption of food rich in carotenoid has been associated with decreased incidence of inflammatory disease and oxidative stress related chronic diseases.

Anthocyanin is considered as one of the largest and most important group of water-soluble pigments present in wide range of fruits and vegetables. Anthocyanin has good antioxidant property that mitigates the damaging effect of oxidative stress.Various studies have demonstrated the beneficial role of anthocyanin on our health. A study by Bellido GG and Beta (2009) had shown the oxygen radical scavenging capacity of anthocyanin. Anthocyanin plays a beneficial role in diabetics by maintaining the lipid profile.

## CONCLUSION

Green chilies which are a very popular spice used by people world-over have multitude beneficial property. The pigments present in chilies change according to the ripeness. But all the pigments haveits own useful property. Chlorophyll which predominates in the initial stage decreases to almost zero in the final stage. The concentration of both carotenoids and anthocyanin increases towards the final stage of study with concentration of anthocyanin predominating. Chili should be consumed in our diet to harness the benefits of this wonderful spice.

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