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*RESEARCH ARTICLE*

# ANALYSIS OF CHLOROPHYLL AND Β-CAROTENE CONTENT OF WINTER VEGETABLES IN BANGLADESH

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**ABSTRACT**

Winter vegetables are a prime source of essential nutrients and biomolecules required for human growth, development and active defense system of the body. The experiment was conducted to determine the chlorophyll and β-carotene content of winter vegetables using the spectrophotometric method. Chlorophyll content in winter vegetables ranged from 0.24 mg/100g in beetroot to 54.91 in radish leaf. The β-carotene content in winter vegetables ranged from 3.07 μg/100g in beetroot to 1060.13 μg/100g in radish leaf. In the cluster analysis,,cluster II included spinach, radish leaf, and goat weed with high chlorophyll and β-carotene content of winter vegetables. Euclidean distance between cabbage and sorrel was the lowest (16.09) while largest distance was observed between beetroot and radish leaf (1059.18). Highly significant positive correlation was observed between β-carotene and chlorophylls. Radish leaf, spinach, goosefoot, roselle leaf and roselle fruit were observed to have the high total chlorophyll and β-carotene content among the studied winter vegetables. They should be regularly included in the diet for adequate supply of protective biomolecules in the winter season for enhanced defense system of the body .

**KEYWORDS**

Plant pigments, spectrophotometry, Cluster analysis, Distance matrix, Correlation coefficient.

## 1. INTRODUCTION

Vegetables are one of the major sources of nutrients, vitamins, fibers and antioxidants for human health (Isabelle et al., 2010; Ullah et al., 2022). Leafy green vegetables are well known for their characteristic flavor, color, and therapeutic value (Faller and Fialho, 2009). These vegetables also contain chlorophylls and carotenoids, which are responsible for visual quality attributes (Xue and Yang, 2009; Kimura and Rodriguez-Amaya, 2002). Plants are unique sources of bioactive compounds required for diseases resistance of our body (Hossain et al.,2020). Dietary intake of chlorophyll and β-carotene is associated with decreased risks of cancer and age-related macular degeneration (Mortensen et al., 2001). Studies indicate that a high intake of a variety of vegetables, providing a mixture of carotenoids, was more strongly associated with reduced cancer and eye disease risk than intake of individual carotenoid supplements (Johnson et al., 2000; Le Marchand et al., 1993).

Chlorophylls are the earth's most important organic molecules as they are necessary for photosynthesis (Markovic et al., 2012). It is a green pigment containing magnesium ions at the center of the porphyrin ring. There are various types of chlorophyll structure (chlorophyll a, chlorophyll b, chlorophyll c, chlorophyll d), but plants contain chlorophyll a and b. Chlorophyll b differs from chlorophyll an only in aldehyde group bonded to the porphyrin, compared to the methyl group for chlorophyll a. Chlorophyll a is a photosynthetic pigment and absorbs blue, red, and violet wavelengths in the visible spectrum, while chlorophyll b absorbs blue light and is used to complete the absorption spectrum of chlorophyll a.

Carotenoids are an important group of pigments in bacteria, algae, and higher plants, where they function as accessory photosynthetic pigments covering regions of the visible spectrum not utilized by chlorophylls. Carotenoids belong to tetraterpenoids, and they have split into two classes: xanthophylls (which contain oxygen) and carotenes (hydrocarbons, containing no oxygen). Chlorophylls and carotenoids have an important role in the prevention of various diseases such as cancer, cardiovascular diseases, and other chronic diseases (Sangeetha and Baskaran, 2010). The study related to pigment content of the winter vegetables of Bangladesh still inadequate at the field conditions. For this reason this study was undertaken to evaluate the pigment content, especially chlorophyll and β-carotene in winter vegetables.

## 2. MATERIAL AND METHODS

### 2.1 Plant Materials and Experimental Design

The experiment was conducted in an open field conditions. During its growing season, all standard growing measures were taken. The experiment consisted of fourteen winter vegetable species. From these three were root vegetables like beetroot, carrot, and red radish; one was fruit vegetable roselle fruit (Table 1) and 10 were leafy vegetables like roselle leaf, garden purslane, cabbage, sorrel, beet spinach, watercress, red radish leaf, hydrocotyle, goosefoot and goat weed (Table 2). Samples for

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the study were collected from the research field of the regional station, Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN), Noakhali, Bangladesh. For the purposes of this research, samples were harvested at the full stage. All the samples were thoroughly cleaned using deionized water to remove adhering contaminants. The vegetables were packed in a 0.5 mm polythene bag and transferred to the lab for nutritional analysis. The nutritional analysis was carried out in the post-harvest laboratory of the Bangladesh Agricultural Research Institute (BARI), Gazipur, and Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during the period from December 2021 to February 2022.The study was conducted following the randomized complete block design with three replications. For the lab study three samples per treatment were analyzed.

### 2.2 Chlorophyll Content

Fresh samples were collected and 0.05g was weighed by using an electronic balance. Homogenize with 10 ml acetone-hexane (4:6 by v/v) solution. After filtering the centrifuge sample. measure optical density using UV-2100 spectrophotometer: 663nm, 645nm, 505nm, and 453 nm. Chlorophyll content was estimated by using the method followed by (Yoshida et al., 1976). Calculated chlorophyll a, and chlorophyll b using the following formula (mg/100 g on fresh wt. basis.

Chlorophyll a = (12.7 x D663)-(2.69 x $D_{645}$) x DF

Chlorophyll b = (22.9 x $D_{645}$)-(4.68 x $D_{663}$) x DF

Where D645 = Absorbance at 645 nm wavelength

D663 = Absorbance at 663 nm wavelength, and 12.7, 2.69, 22.9, and 4.68 are absorbance co-efficient.

DF = Dilution factor

### 2.2 B-Carotene Content

One day before preparing a mixture solvent acetone:hexone (2:3) by using 40 mL Acetone and 60 mL hexane to 100 mL mixture solvent and then placed the solvent in the refrigerator. The sample was homogenized with a warring blender. Taken 10g homogenized sample tissue in 50 mL graduated orange cap tubes. Then added 16 mL of the cold acetone-hexane mixture and placed the caps tightly. Vertexing the tubes for 60 seconds and then placed in the freezer (-30$^0$C). Samples were then left at room temperature for five minutes to separate both phases, i.e. the polar and non-polar phases. The supernatant (only hexane) was transferred in another tube (extraction pool), using a plastic pauster pipette, and put the cap. The absorbance of the filtrate was measured at 663 nm, 645 nm, 505 nm, and 453 nm by spectrophotometer at the same time. β-carotene content was determined as per the procedure prescribed by Nagata and Yamashita, 1992 (Nagata and Yamashita, 1992). β-carotene was calculated using the following formula (mg/100g) on fresh wt. basis.

β-carotene = ((0.216*A663) - (1.22*A645) - (0.304*A505) + (0.452*A453)* (10/0.1042)

### 2.4 Statistical Analysis

The mean and standard deviation (S.D) of chlorophyll and β-carotene content for the samples was performed using the computer software microsoft excel. Cluster analysis (CA), a multivariate technique, was done with the purpose of classifying the objects of the system into categories or clusters based on their similarities (Ogwu and Osawaru, 2016). Cluster analysis was done by means of Ward's method using Euclidean distances as a measure of similarity. Ward's method attempts to minimize the sum of squares of any two (hypothetical) clusters that can be formed at each step. Euclidean distance is most common way to measure distance between objects.

Distance (x,y)= $\sqrt{\sum_{i=1}^{n}(x_i - y_i)^2}$

The distances can be affected by differences in scale among the dimensions from which the distances are computed, so the variables must be standardized. The principal component analysis and distance matrix were performed by using software OPSTAT (Sheoran et al., 1998).

**Table 1:** List of vegetables selected for chlorophyll and β-carotene determination

| Sl No | Name | Type | Scientific Name | Family | Parts use |
|---|---|---|---|---|---|
| 01 | Beet | Root vegetable | *Beta vulgaris* | Amaranthaceae | Root |
| 02 | Carrot | | *Daucus carota* | Apiaceae | Root |
| 03 | Red Radish | | *Raphanus sativus* | Cruciferae | Root |
| 04 | Roselle fruit | Fruit Vegetable | *Hibiscus sabdariffa* | Malvaceae | Fruit |
| 05 | Roselle leaf | Leafy vegetables | *Hibiscus sabdariffa* | Malvaceae | Leaf, fruit |
| 06 | Garden purslane | | *Portulaca oleracea* | Portulacaceae | whole plant |
| 07 | Cabbage | | *Brassica oleracea var capitata* | *Cruciferae* | whole plant |
| 08 | Red radish leaf | | *Raphanus sativus* | Cruciferae | Leaf, root |
| 09 | Sorrel | | *Rumex vasicarious* | Polygonaceae | whole plant |
| 10 | Spinach | | *Spinacea oleracea* | Amaranthaceae | whole plant |
| 11 | Hydrocotyle | | *Hydrocotyle asiatica* | Mackinlayaceae | whole plant |
| 12 | Goose foot | | *Chenopodium album* | Chenopodiaceae | Leaf |
| 13 | Water cress | | *Enhydra fluctuans* | Asteraceae | whole plant |
| 14 | Goat weed | | *Blumea lacera* | Asteraceae | Leaf |

## 3. Results and Discussion

Analyses performed on winter vegetables showed a large variety of chlorophyll a, b and β-carotene content. Chlorophyll and β-carotene content in vegetables was measured in fresh condition. The amount of chlorophyll a was higher than chlorophyll b and β-carotene in all selected vegetables except roselle fruit, spinach and goat weed, which is in agreement with the literature (López-Ayerra et al., 1998). Variations in chlorophyll a, chlorophyll b, and total chlorophyll among the winter vegetable ranged between 0.17 to 27.20 mg/100g, 0.07 to 29.76 mg/100g, and 0.24 to 54.91 mg/100g of fresh weight, respectively. The result showed Goosefoot has maximum chlorophyll a, roselle fruit has maximum chlorophyll b, and spinach has maximum total chlorophyll content from the studied winter vegetables. The lowest chlorophyll a, b, and the total are found in the winter vegetable beetroot. These results showed that the chlorophyll concentration in the vegetables is dependent on species. In the present investigation, provitamin A (β-carotene) content ranges from 3.07 to 1060.13 µg/100g. The highest β-carotene content in radish leaf (1060.13 µg/100g) was three hundred times higher than the lowest content in beetroot (3.07 µg/100g). The result presented in the study has shown that β-carotene content varied widely among vegetables indicating the need for different servings of these foods. Among the food samples analyzed, radish leaf had the highest content of β-carotene.

### 3.1 Roots and Fruits Vegetables

A result is presented in Table 3 for the determination of chlorophyll and β-carotene content in selected roots and fruits vegetable. The highest content of chlorophyll a was found in roselle fruit (4.28 mg/100g) which was followed by carrot (1.97 mg/100g). The lowest chlorophyll a was observed in beetroot (0.17 mg/100g). (Table 3). The highest amount also was found in roselle fruit for chlorophyll b and the lowest also in beetroot (0.07 mg/100g). The total chlorophyll was observed for the highest in the vegetable roselle fruit (34.04 mg/100g). The content of chlorophyll a in samples decreased in the order of Roselle fruit > Carrot > Red Radish > Beetroot. Chlorophyll b content in the samples decreased in the order roselle fruit > Carrot > Red Radish > Beetroot. β-Carotene varied in roots and fruits vegetables ranging from 3.07 µg/100g in beetroot to 630.98 µg/100g in carrot.

**Table 2:** Chlorophyll and β-carotene content of selected root and fruit vegetables

| Sl No | English Name | chlorophyll a (mg/100g) | chlorophyll b (mg/100g) | Total chlorophyll (mg/100g) | β-Carotene (μg/100g) |
|---|---|---|---|---|---|
| 01 | Beetroot | 0.17±0.003 | 0.07±0.017 | 0.24±0.014 | 3.07±0.149 |
| 02 | Carrot | 1.97±0.131 | 0.87±0.057 | 2.84±0.137 | 630.98±3.715 |
| 03 | Red Radish | 0.86±0.004 | 0.48±0.057 | 1.35±0.060 | 106.47±1.543 |
| 04 | Roselle fruit | 4.28±0.007 | 29.76±0.237 | 34.04±0.244 | 35.54±0.806 |
| | Min. | 0.17±0.003 | 0.07±0.017 | 0.24±0.014 | 3.07±0.149 |
| | Max. | 4.28±0.007 | 29.76±0.237 | 34.04±0.244 | 630.98±3.715 |

*Each value represents the mean ± SD of three determinations on wet weight (WW) basis.*

### 3.2 Leafy Vegetables

In the case of leafy vegetables, the highest content of chlorophyll a was recorded in goosefoot (27.20 mg/100g) which was followed by spinach (26.55 mg/100g), radish leaf (26.52 mg/100g) and roselle leaf (23.47 mg/100g) (Table 3). The highest chlorophyll b content was observed in radish leaf (28.39 mg/100g) which was followed by spinach (27.73 mg/100g) and goat weed (26.56 mg/100g). The lowest content of chlorophyll b was found in sorrel (4.67 mg/100g). In green vegetables, chlorophyll degradation will occur along with leaf senescence after harvest (Ferrante et al., 2004; X. Zhang et al., 2011). The record investigated the content of chlorophyll a, b in spinach and found chlorophyll a was from 51.4 to 6.36 mg/100g and chlorophyll b content from 92.3 to 114.2 mg/100g, which are lower than the values we found in our work (Andrejiová and Mendelová, 2012) Andrejiová, Mendelová, 2012). Larsen and Christensen (2005) found high levels of chlorophyll in the cabbage (Larsen and Christensen, 2005). Kopsell *et al.* (2004) found that chlorophyll content in fresh spinach is 270.00 to 488.00 mg/100g as dry weight, depending on the variety and the year of production (Kopsell et al., 2004). The chlorophyll content in our samples of spinach on a wet basis was 26.55 mg/100g. Chlorophyll b was 27.73 mg/100g wet basis. Spinach is one of the most nutritionally important species of leafy vegetables. A major contributor to the total carotene content of vegetables is β-carotene which is the main carotenoid with pro-vitamin A activity (Olson, 2004). Regarding the concentration of this pigment. Podsedek (2007) reported that leafy vegetables are indeed a richer source of β-carotene than other crops (Podsędek, 2007). Bhaskarachary *et al.* (2008) also demonstrated similar domination of β-carotene in 17 species of leafy vegetables (Kandlakunta et al., 2008).

Among the leafy vegetables analyzed in our study, radish leaf had the highest content of β-carotene (1060.13 μg/100g fwt), followed by goat weed (964.84 μg/100g fwt), spinach (887.44 μg/100g fwt), goosefoot (563.21 μg/100g fwt) and roselle leaf (350.48 μg/100g fwt). The values in our study are in agreement with the data of Sangeetha and Baskaran (2010) who analyzed the β-carotene content in several vegetables (Sangeetha and Baskaran, 2010). Pritwani and Mathur (2017) reported that spinach leaves (*Spinacia oleracea*) had β-carotene with the average value 3468 μg/100g that was higher than the present study (Pritwani and Mathur, 2017).

**Table 3:** Chlorophyll and β-carotene content of selected leafy vegetables

| Sl No | English Name | chlorophyll a (mg/100g) | chlorophyll b (mg/100g) | Total chlorophyll (mg/100g) | β-Carotene (μg/100g) |
|---|---|---|---|---|---|
| 01 | Roselle leaf | 23.47±0.252 | 16.91±0.503 | 40.38±0.698 | 350.48±2.413 |
| 02 | Garden purslane | 16.60±0.266 | 7.62±0.488 | 24.22±0.703 | 242.26±1.593 |
| 03 | Cabbage | 14.09±0.176 | 7.42±0.449 | 21.51±0.385 | 180.24±1.292 |
| 04 | Sorrel | 6.56±0.290 | 4.67±0.469 | 11.23±0.385 | 170.30±0.784 |
| 05 | Spinach | 26.55±0.402 | 27.73±0.537 | 54.28±0.602 | 887.44±1.873 |
| 06 | Water Cress | 17.45±0.339 | 8.60±0.551 | 26.05±0.442 | 300.32±1.851 |
| 07 | Radish leaf | 26.52±0.423 | 28.39±0.598 | 54.91±0.795 | 1060.13±2.036 |
| 08 | Hydrocotyle | 22.69±0.853 | 15.31±0.754 | 38.00±0.799 | 104.66±1.762 |
| 09 | Goosefoot | 27.20±0.412 | 22.77±0.543 | 49.97±0.410 | 563.21±2.168 |
| 10 | Goat weed | 3.93±0.715 | 26.56±0.594 | 30.48±0.616 | 964.84±2.010 |
| | Min. | 3.93±0.715 | 4.67±0.469 | 11.23±0.385 | 104.66±1.762 |
| | Max. | 27.20±0.412 | 28.39±0.598 | 54.91±0.795 | 1060.13±2.036 |

*Each value represents the mean ± SD of three determinations on wet weight (WW) basis.*

### 3.3 Principal Component Analysis

Principal component analysis was carried out to establish a clear relationship between the different vegetable species in phytochemicals or quality parameters and it was an efficient technique to classify the studied vegetables samples according to the pigment content (Alam et al., 2020). With respect to eigen values (> 1), one principal component was obtained with their factor loadings (Table 4). 72.9 percent of the original variance in the data set of quality compound (PC1 72.9%) was explained by the first one principal component (Table 5). Similar findings were also confirmed in other previous studies (Haque et al., 2022; Zahir Ullah et al., 2022).The one PC explained 72.9% of the x-variables selecting four parameters, including the Chlorophyll a (r= 0.480), chlorophyll b (r=0.517), total chlorophyll (r=0.573) and β-carotene (r=0.417) were mainly accounted for with PC1, while chlorophyll a (r=-0.579), β-carotene (r=0.767) were mainly accounted for with PC2 as shown in Table 6.

**Table 4:** Eigenvalues of Correlation Matrix

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| Eigenvalues | **2.917** | 0.673 | 0.410 |
| Proportion | 0.729 | 0.168 | 0.102 |
| Cumulative Proportion | 0.729 | 0.898 | 1.000 |

**Table 5:** Eigenvalues of the principal components of the correlation matrix for 14 winter vegetables species

| Principal component | Eigenvalue | Difference between Eigenvalue | % Variation explained | Cumulated value |
|---|---|---|---|---|
| 1 | **2.917** | 2.244 | 0.729 | 0.729 |
| 2 | 0.673 | 0.263 | 0.673 | 0.898 |
| 3 | 0.410 | 0.410 | 0.410 | 1.000 |

| **Table 6:** Loadings (Eigenvectors) of Correlation Matrix | | | | |
|---|---|---|---|---|
| Traits | PC1 | PC2 | PC3 | PC4 |
| Chlorophyll a | **0.480** | **-0.579** | **0.499** | **-0.431** |
| Chlorophyll b | **0.517** | 0.166 | **-0.702** | **-0.461** |
| Total chlorophyll | **0.573** | -0.223 | -0.140 | **0.776** |
| β-carotene | **0.417** | **0.767** | **0.488** | 0.000 |

### 3.4 Cluster Analysis

Chemometrics is the branch of chemistry dealing with the analysis of chemical data (extracting information from data) and ensuring that experimental data contain maximum information (Wold, 1995). Chemometrics is used to classify of food products based on their main compounds (Woodcock et al., 2007). In cluster analysis (CA), samples are grouped based on similarities (Alam et al., 2016; Yong et al., 2006). In this study, CA was performed on the standardized data, on chlorophyll a, chlorophyll b, and β-carotene content in fresh vegetables species. Four clusters were formed after applying cluster analysis to pigment content in fresh vegetables. Beetroot, red radish, roselle fruit, hydrocotyle were under cluster I (Figure 1) with less chlorophyll and β-carotene. In cluster II included spinach, radish leaf, and goat weed with high chlorophyll and β-carotene content. Carrot, Goosefoot formed cluster III with medium chlorophyl and β-carotene content, while roselle leaf, garden purslane, cabbage, sorrel, and watercress formed cluster IV (Table 7) with medium chlorophyll and less β-carotene content.

| **Table 7:** Distribution of 14 vegetables species in different clusters | | |
|---|---|---|
| Cluster | No. of Species | Name of Species |
| Cluster I | 4 | Beet root, Red Radish, Roselle fruit, Hydrocotyle |
| Cluster II | 3 | Spinach, Radish leaf, Goat weed |
| Cluster III | 2 | Carrot, Goosefoot |
| Cluster IV | 5 | Roselle leaf, Garden purslane, Cabbage, Sorrel, Water Cress |
| Total | 14 | |

### 3.5 Distance Matrix

Cabbage and sorrel showed the most similar pigment content, so it was expected that they are "nearest neighbors". Euclidean distance between these two species was the lowest (16.09) (Table 8). The largest pigment distance was observed between beetroot and radish leaf (1059.18), and accordingly, differences between their pigment content were highest (Table 8).

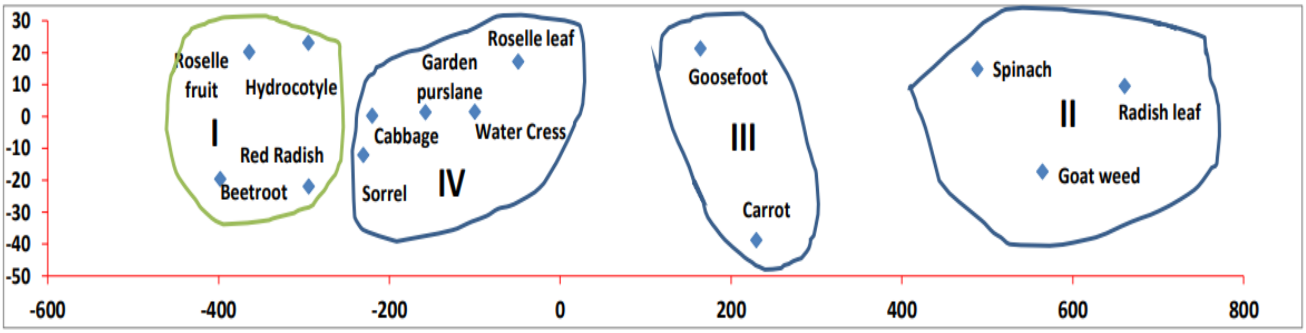


**Figure 1:** Distribution of vegetables species in different cluster based on PC I and PC II

| **Table 8:** Euclidean distances among winter vegetables species | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Crops** | **Carrot** | **Red Radish** | **Roselle fruit** | **Roselle leaf** | **Garden purslane** | **Cabbage** | **Sorrel** | **Spinach** | **Water Cress** | **Radish leaf** | **Hydrocotyle** | **Goosefoot** | **Goat weed** |
| **1** | **Beet root** | **627.90** | **103.41** | **52.31** | **350.86** | **240.97** | **179.06** | **167.77** | **886.84** | **298.91** | **1059.18** | **111.65** | **563.43** | **962.55** |
| **2** | **Carrot** | | **524.50** | **596.51** | **284.27** | **389.63** | **451.34** | **460.79** | **264.08** | **331.91** | **433.87** | **528.10** | **89.05** | **335.62** |
| **3** | **Red Radish** | | | **81.13** | **248.65** | **138.65** | **77.80** | **64.97** | **783.65** | **196.19** | **955.91** | **45.04** | **460.60** | **859.18** |
| **4** | **Roselle fruit** | | | | **314.92** | **206.89** | **145.32** | **137.41** | **852.17** | **264.80** | **1024.81** | **69.20** | **527.93** | **929.28** |
| **5** | **Roselle leaf** | | | | | **110.02** | **171.80** | **183.68** | **537.25** | **53.14** | **709.89** | **245.84** | **213.06** | **614.59** |
| **6** | **Gardenpurslane** | | | | | | **62.10** | **73.70** | **646.25** | **58.10** | **818.76** | **138.62** | **322.50** | **722.73** |
| **7** | **Cabbage** | | | | | | | **16.09** | **708.36** | **120.20** | **880.86** | **78.23** | **384.56** | **784.78** |
| **8** | **Sorrel** | | | | | | | | **719.08** | **131.27** | **891.44** | **73.43** | **395.76** | **794.96** |
| **9** | **Spinach** | | | | | | | | | **588.16** | **172.69** | **783.05** | **324.29** | **82.91** |
| **10** | **Water Cress** | | | | | | | | | | **760.66** | **196.19** | **264.53** | **664.65** |
| **11** | **Radish leaf** | | | | | | | | | | | **955.72** | **496.97** | **99.91** |
| **12** | **Hydrocotyle** | | | | | | | | | | | | **458.79** | **860.34** |
| **13** | **Goosefoot** | | | | | | | | | | | | | **402.46** |
| **Min: 16.094, Max: 1059.175, Mean: 414.265, VAR: 94598.395, SD: 298.861, SE: 172.548, CV: 72.143** | | | | | | | | | | | | | | |

### 3.6 Correlation

To determine the relationships among the analyzed traits, a Pearson correlation coefficient analysis was performed as shown in Table 9. Highly significant correlation was observed between chlorophyll a and chlorophyll b (r=0.514**), chlorophyll a and total chlorophyll (0.861**), and significant correlation with β-carotene (0.385*). Chlorophyll b performed highly significant correlation with total chlorophyll (0.879**) and β-carotene (0.573**). At the same time, total chlorophyll was observed highly significant correlation with β-carotene (0.554**) (Table 9). Similarities in behavior of carotenoids and chlorophylls have been reported for other crop species (Grunwald et al., 1977; Terry and Abadía, 1986). Ihl *et al.* (1994) found chlorophylls to highly correlated with total carotenoid levels in the leaves of Swiss chard (*Beta vulgaris* L.)(Ihl et al., 1994). Our results support these correlative relationships among these winter vegetables (Table 9). This suggests it may be possible to use chlorophyll content, or green coloration, to estimate gross β-carotene concentration in green vegetables. The positive correlation between the contents of chlorophyll and β-carotene has been also reported for other leafy crop species, like as kale, Swiss chard, and lettuce (Caldwell and Britz, 2006; Kopsell et al., 2004; Ihl et al., 1994).

**Table 9:** Pearson Correlation Matrix among chlorophyll and β-carotene content

| Traits | Chlorophyll a | Chlorophyll b | Total chlorophyll | β-carotene |
|---|---|---|---|---|
| Chlorophyll a | 1.000 | 0.514** | 0.861** | 0.385* |
| Chlorophyll b | | 1.000 | 0.879** | 0.573** |
| Total chlorophyll | | | 1.000 | 0.554** |
| β-carotene | | | | 1.000 |

### 3.7 Retention of Chlorophyll

The cooking process can cause changes in the physical characteristics and chemical composition of vegetables (Zhang and Hamauzu, 2004). Chlorophylls are known to be easily degraded by acids, heat, and light (Tonucci and Von Elbe, 1992). Food color play's an important role in product acceptability, so it is important to prevent chlorophyll loss. The reason for the green color loss during processing is attributed to the conversion of chlorophylls to pheophytins. Turkmen *et al.* (2006) reported that chlorophyll a+b content in boiled spinach and broccoli was lower than in fresh vegetables (Turkmen et al., 2006). The reduction of chlorophyll a and b content is attributed to the degradation of chlorophylls in their main derivatives pheophytin a and b, respectively (Van Boekel, 1999). Pheophytin formation is the result of the $Mg^{2+}$ elimination of chlorophyll. Pheophytin can be produced by an acidic cooking environment or by prolonged cooking. So, we can consume food as raw stage as salads, paste, drinks, light heating with oil. As salads we can consume beet spinach, roselle fruits, roselle leaf, carrot, beet, red radish, cabbage, hydrocotyle and sorrel.

## 4. Conclusion

Chlorophyll a, b, and carotenoid content varied between species. Chlorophylls and β-carotene have a significant role in the human diet, so it is important to determine commonly consumed winter vegetables. In distance matrix cabbage and sorrel showed the most similar pigment content and the largest pigment distance was observed between beetroot and radish leaf. In Pearson correlation, it was observed that highly significant positive correlation between β-carotene and chlorophylls. Cluster analysis can be used to classify vegetables according to pigment content of winter vegetables. Cluster II included spinach, radish leaf, and goat weed with high chlorophyll and β-carotene content of winter vegetables. According to pigment content in fresh vegetables, four statistically significant clusters were obtained. The data generated on the composition of carotenoids and chlorophylls in winter vegetables could be the basis for suggesting the inclusion of these leafy vegetables in a daily diet to overcome health problems, such a provitamin A deficiency. Therefore, it is evident from this investigation that the selection of specific vegetables species that are rich in nutritional composition is important to improve the nutrient intake in the diet. Based on these results, radish leaf, goat weed, spinach, goosefoot, carrot, roselle leaf, and roselle fruit can be used in mixed fresh cut salads or they can be consumed as whole product. To get more precise result of the studied pigment containment, future study should include more vegetables species especiallyindigenous and underutilized vegetables of Bangladesh.

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