

ANALYSIS OF CHLOROPHYLL AND B-CAROTENE CONTENT OF WINTER VEGETABLES

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ABSTRACT

As a primary pigment of leafy green vegetables, chlorophyll plays a major role in indicating vegetable growth status. Carotenoids are natural fat-soluble pigments that are common in colorful plants. They act as provitamin A and are beneficial for human health, especially for vision. An experiment was conducted to determine the chlorophyll and β -carotene content of winter vegetables. The chlorophyll and β -carotene content of fresh vegetables was determined using the spectrophotometer method. Chlorophyll b content in winter vegetables ranged from 0.07 mg/100 g in beetroot to 29.76 in roselle fruit. It was found that radish leaf (54.91 \pm 0.795 mg/100 g) is very rich in total chlorophyll followed by spinach (54.28 mg/100 g), goosefoot (49.97 mg/100 g), roselle leaf (40.38 mg/100 g), and roselle fruit (34.04 mg/100 g) among the studied winter vegetables. The β -carotene content in winter vegetables ranged from 3.07 μ g/100 g in beetroot to 1060.13 μ g/100 g in radish leaf. This study has shown that radish leaf (1060.13 μ g/100 g) is very rich in β -carotene followed by goat weed (964.84 μ g/100 g), spinach (887.44 μ g/100 g), carrot (630.98 μ g/100 g), and goosefoot (563.21 μ g/100 g). 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 the largest distance was observed between beetroot and radish leaf (1059.18). A highly significant positive correlation was observed between β -carotene and chlorophylls. Radish leaf, spinach, goosefoot, roselle leaf, and roselle fruit were observed to have a high total chlorophyll content as well as radish leaf, goat weed, spinach, carrot, and goosefoot were observed to have high β -carotene content among the studied winter vegetables. They should be regularly included in the diet for the adequate supply of chlorophyll and β -carotene in the winter season for the protection of our health from diseases.

Keywords: Chlorophyll, β -carotene, Vegetables, Cluster analysis, Distance matrix, Correlation coefficient.

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INTRODUCTION

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 (Kimura and Rodriguez-Amaya, 2002), which are responsible for visual quality attributes (Xue and Yang, 2009).

Chlorophylls are the earth's most important organic molecules as they are necessary for photosynthesis (Marković *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 a only in the 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). This study was undertaken to evaluate pigment content, especially chlorophyll and β -carotene in winter vegetables. Cluster analysis (CA) was applied to group vegetables based on their pigment content.

METHODS

The investigation was carried out in the post-harvest laboratory, Bangladesh Agricultural Research Institute, Gazipur, and the

Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during the period from December 2021 to February 2022.

Plant material

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

Chlorophyll content

Fresh samples were collected and 0.05 g 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: 663 nm, 645 nm, 505 nm, and 453 nm. Chlorophyll content was estimated by using the method followed by Yoshida *et al.* (1996). Calculated chlorophyll a and chlorophyll b using the following formula (mg/100 g on fresh wt. basis).

$$\text{Chlorophyll a} = (12.7 \times D_{663}) - (2.69 \times D_{645}) \times DF$$

$$\text{Chlorophyll b} = (22.9 \times D_{645}) - (4.68 \times D_{663}) \times DF$$

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

S. No.	Name	Type	Scientific name	Family	Parts use
01	Beet	Root vegetable	<i>Beta vulgaris</i>	<i>Amaranthaceae</i>	Root
02	Carrot		<i>Daucus carota</i>	<i>Apiaceae</i>	Root
03	Red radish		<i>Raphanus sativus</i>	<i>Cruciferae</i>	Root
04	Roselle fruit	Fruit vegetable	<i>Hibiscus sabdariffa</i>	<i>Malvaceae</i>	Fruit
05	Roselle leaf	Leafy vegetables	<i>Hibiscus sabdariffa</i>	<i>Malvaceae</i>	Leaf, fruit
06	Garden purslane		<i>Portulaca oleracea</i>	<i>Portulacaceae</i>	Whole plant
07	Cabbage		<i>Brassica oleracea var capitata</i>	<i>Cruciferae</i>	Whole plant
08	Red radish leaf		<i>Raphanus sativus</i>	<i>Cruciferae</i>	Leaf, root
09	Sorrel		<i>Rumex vasicarius</i>	<i>Polygonaceae</i>	Whole plant
10	Spinach		<i>Spinacea oleracea</i>	<i>Amaranthaceae</i>	Whole plant
11	Hydrocotyle		<i>Hydrocotyle asiatica</i>	<i>Mackinlayaceae</i>	Whole plant
12	Goosefoot		<i>Chenopodium album</i>	<i>Chenopodiaceae</i>	Leaf
13	Watercress		<i>Enhydra fluctuans</i>	<i>Asteraceae</i>	whole plant
14	Goat weed		<i>Blumea lacera</i>	<i>Asteraceae</i>	Leaf

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

S. No.	English name	chlorophyll A (mg/100 g)	chlorophyll B (mg/100 g)	Total chlorophyll (mg/100 g)	β -carotene (μ g/100 g)
01	Beetroot	0.17 \pm 0.003	0.07 \pm 0.017	0.24 \pm 0.014	3.07 \pm 0.149
02	Carrot	1.97 \pm 0.131	0.87 \pm 0.057	2.84 \pm 0.137	630.98 \pm 3.715
03	Red Radish	0.86 \pm 0.004	0.48 \pm 0.057	1.35 \pm 0.060	106.47 \pm 1.543
04	Roselle fruit	4.28 \pm 0.007	29.76 \pm 0.237	34.04 \pm 0.244	35.54 \pm 0.806
	Min.	0.17 \pm 0.003	0.07 \pm 0.017	0.24 \pm 0.014	3.07 \pm 0.149
	Max.	4.28 \pm 0.007	29.76 \pm 0.237	34.04 \pm 0.244	630.98 \pm 3.715

Each value represents the mean \pm SD of three determinations on a wet weight (WW) basis

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

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

Each value represents the mean \pm SD of three determinations on wet weight (WW) basis

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.

β -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 10 g 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 s and then placed in the freezer (-30°C). Samples were then left at room temperature for 5 min to separate both phases, i.e., the polar and non-polar phases. Placed the supernatant (only hexane) in another tube (extraction pool), using a plastic pasteur 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 *et al.*, 1992. β -carotene was calculated using the following formula (mg/100 g) on fresh wt. basis.

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

$$\beta\text{-carotene}=(0.216 \times A663)-(1.22 \times A645)-(0.304 \times A505) + (0.452 \times A453) \times (10/0.1042)$$

Statistical analysis

The mean and standard deviation of chlorophyll and β -carotene content for the samples was performed using the computer software Microsoft Excel. CA is a multivariate technique, with the purpose of classifying the objects of the system into categories or clusters based on their similarities (Richard and Dean, 2002). CA 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 the most common way to measure distance between objects.

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

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

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 the software OPSTAT (Sheoran *et al.*, 1998).

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 (Lopez-Ayerra *et al.*, 1998). Variations in chlorophyll a, chlorophyll b, and total chlorophyll among the winter vegetable ranged between 0.17 and 27.20 mg/100 g, 0.07–29.76 mg/100 g, and 0.24–54.91 mg/100 g 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 $\mu\text{g}/100\text{ g}$. The highest β -carotene content in radish leaf (1060.13 $\mu\text{g}/100\text{ g}$) was 300 times higher than the lowest content in beetroot (3.07 $\mu\text{g}/100\text{ g}$). 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.

Roots, fruits and 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/100 g) which was followed by carrot (1.97 mg/100 g). The lowest chlorophyll a was observed in beetroot (0.17 mg/100 g) (Table 3). The highest amount also was found in roselle fruit for chlorophyll b and the lowest also in beetroot (0.07 mg/100 g). The total chlorophyll was observed for the highest in the vegetable roselle fruit (34.04 mg/100 g). 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 of roselle fruit>Carrot>Red Radish>Beetroot. β -Carotene varied in roots, fruits and vegetables ranging from 3.07 $\mu\text{g}/100\text{ g}$ in beetroot to 630.98 $\mu\text{g}/100\text{ g}$ in carrot.

Leafy vegetables

In the case of leafy vegetables, the highest content of chlorophyll a was recorded in goosefoot (27.20 mg/100 g) which was followed by spinach (26.55 mg/100 g), radish leaf (26.52 mg/100 g) and roselle leaf (23.47 mg/100 g) (Table 3). The highest chlorophyll b content was observed in radish leaf (28.39 mg/100 g) which was followed by spinach (27.73 mg/100 g) and goat weed (26.56 mg/100 g). The lowest content of chlorophyll b was found in sorrel (4.67 mg/100 g). In green vegetables, chlorophyll degradation will occur along with leaf senescence after harvest (Antonio *et al.*, 2004; Xuelian *et al.*, 2011). Andrejiová and Mendelová (2012) investigated the content of chlorophyll a, b in spinach and found chlorophyll a was from 51.4 to 6.36 mg/100 g and chlorophyll b content from 92.3 to 114.2 mg/100 g,

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

Table 7: Distribution of 14 vegetable 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, Watercress
Total	14	

which are lower than the values we found in our work. Larsen and Christensen (2005) show high levels of chlorophyll in the cabbage. Kopsell *et al.* (2004) found that chlorophyll content in fresh spinach is 270.00 to 488.00 mg/100 g as dry weight, depending on the variety and the year of production. The chlorophyll content in our samples of spinach on a wet basis was 26.55 mg/100 g. Chlorophyll b was 27.73 mg/100 g 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 provitamin A activity (Olson, 1994). Regarding the concentration of this pigment, Podsedek (2007) reported that leafy vegetables are indeed a richer source of β -carotene than other crops. Bhaskarachary *et al.* (2008) also demonstrated similar domination of β -carotene in 17 species of leafy vegetables.

Among the leafy vegetables analyzed in our study, radish leaf had the highest content of β -carotene (1060.13 $\mu\text{g}/100\text{ g}$ fwt), followed by goat weed (964.84 $\mu\text{g}/100\text{ g}$ fwt), spinach (887.44 $\mu\text{g}/100\text{ g}$ fwt), goosefoot (563.21 $\mu\text{g}/100\text{ g}$ fwt) and roselle leaf (350.48 $\mu\text{g}/100\text{ g}$ 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. Pritwani and Mathur (2017) reported that spinach leaves (*Spinacia oleracea*) had β -carotene with an average value of 3468 $\mu\text{g}/100\text{ g}$, which was higher than the present study.

Principal component analysis

Principal component analysis was carried out to establish a clear relationship between the different vegetable species in phytochemicals or quality parameters. With respect to eigen values >1, one principal component was obtained with their factor loadings (Table 4). 72.9% of the original variance in the data set of quality compound (PC1 72.9%) was explained by the first one principal component (Table 5). 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.

CA

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 food products based on their main compounds (Woodcock et al., 2007). In CA, samples are grouped based on similarities. In this study, CA was performed on the standardized data, on chlorophyll a, chlorophyll b, and β-carotene content in fresh vegetable species. Four clusters were formed after applying CA to pigment content in fresh vegetables. Beetroot, red radish, roselle fruit, and hydrocotyle were under cluster I (Fig. 1) with less chlorophyll and β-carotene. Cluster II included spinach, radish leaf, and goat weed with high chlorophyll and β-carotene content. Carrot and Goosefoot formed cluster III with medium chlorophyll 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.

Distance matrix

Cabbage and sorrel showed the most similar pigment content, so it was expected that they were “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).

Correlation

To determine the relationships among the analyzed traits, a Pearson correlation coefficient analysis was performed as shown in Table 9. A highly significant correlation was observed between chlorophyll a and chlorophyll b ($r=0.514^{**}$), chlorophyll a and total chlorophyll (0.861^{**}), and a significant correlation with β-carotene (0.385^{*}). Chlorophyll b performed a 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 the behavior of carotenoids and chlorophylls have been reported for other crop species (Grunwald et al., 1977; Terry and Abadia, 1986). Ihl et al. (1994) found chlorophylls to be highly correlated with total carotenoid levels in the leaves of Swiss chard (*Beta vulgaris* L.). 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, such as kale (Kopsell et al., 2004), Swiss chard (Ihl et al., 2006), and lettuce (Caldwell and Britz, 2006).

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 plays 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. The reduction of chlorophyll a and b content is attributed to the degradation of chlorophylls in their main derivatives pheophytin a and b, respectively (Boekel, 1999). Pheophytin formation is the result of the Mg²⁺ elimination of chlorophyll. Pheophytin can be produced by an acidic cooking environment or by prolonged cooking. Hence, we can consume food as raw stage as salads, paste, drinks, and light heating with oil. As salads, we can consume beet spinach, roselle fruits, roselle leaf, carrot, beet, red radish, cabbage, hydrocotyle, and sorrel.

Health benefit

Dietary intake of chlorophyll and β-carotene is associated with decreased risks of cancer and age-related macular degeneration

Table 8: Euclidean distances among winter vegetable species

S. No.	Crops	Carrot	Red radish	Roselle fruit	Roselle leaf	Garden purslane	Cabbage	Sorrel	Spinach	Watercress	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	Garden purslane						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	Watercress										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

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