DOI: http://doi.org/10.5281/zenodo.7011247
U.S. ISSN 2694 -4812

Determination of antioxidants (vitamin C and lycopene) content of winter vegetables

Mohammad Zahir Ullah[1]*, Md Samsuzzaman[2], Md.Sarowar Alam[2], Prince Biswas[3], Md Maksudul Haque[3]

[1]Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN), Regional Research Station, Noakhali, Bangladesh
[2]Regional Agricultural Research Station, Bangladesh Agricultural Research Institute, Moulvibazar, Bangladesh-3210
[3]Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN), Head Office, Araihazar, Narayangonj, Bangladesh
* Corresponding author email: zahirsau@gmail.com
Received: 12 July 2022; Accepted: 19 August 2022

Abstract: **Vegetables are a prime source of antioxidants which play very important role for preventing different chronic diseases in our body. Therefore, an experiment was carried out to determine the antioxidants like vitamin C and lycopene content of winter vegetables. Ascorbic acid content of fresh vegetables was determined by titration method and lycopene content was determined using spectrophotometer method. The ascorbic acid content in root and fruit winter vegetables ranged from 7.13±0.907 mg/100g in beet root to 27.48±1.45 in black tomato. In the leafy vegetables, ascorbic acid content ranged from 11.40±1.664 mg/100g in cabbage to 19.71±1.429 mg/100g in sorrel. It was found that black tomato ((27.48±1.45 mg/1oog)) is very rich in ascorbic acid followed by cherry tomato (26.04±1.12 mg/100g), red tomato (21.36±0.954 mg/100g), sorrel (19.71±1.429 mg/100g), hydrocotyle (17.76±1.264 mg/100g), spinach (17.71±1.532 mg/100g) and roselle leaf (17.36±0.844 mg/100g) among the studied winter vegetables. The lycopene content in root and fruit winter vegetables ranged from 25.25±3.08 µg/100g in beet root to 6648.34±9.89 µg/100g in cherry tomato. In the leafy vegetables, lycopene content ranged from 13.45±4.04 µg/100g in hydrocotyle to 673.98±5.76 µg/100g in roselle leaf. This study has shown that cherry tomato (6648.34±9.89 µg/100g) is very rich in lycopene followed by red tomato (4107.27±8.13 µg/100g), black tomato (1365.21±4.26 µg/100g) and carrot (65.48±4.33 µg/100g). Roselle leaf (673.98±5.76 µg/100g), goat weed (551.18±6.02 µg/100g), spinach (474.19±4.63 µg/100g) were observed to have the high lycopene content among the studied leafy vegetables. Black tomato, red tomato, cherry tomato, roselle leaf and spinach were observed to have the high vitamin C and lycopene content among the studied winter vegetables. They should be regularly included in the diet for adequate supply of vitamin C and lycopene in the winter season.**

***Keywords*— Antioxidant, Vitamin C, Lycopene, Vegetables, Immunity, Cancer**

I. INTRODUCTION

Vegetables are a major sources of antioxidants, such as phenolics, lycopene, ascorbic acid, tocopherol, beta-carotene, flavonoids, and tannins etc., (Isabella et al., 2010; Salah et al., 1995) and consumption of different types of vegetables reduce the risk of chronic diseases such as cancers, heart and neuro diseases (Gerber et al., 2002; Kris-Etherton et al., 2002; Di Matteo & Esposito, 2003). Epidemiological evidence linked with eating diet rich in fresh fruits and vegetables for protection against chronic degenerative diseases (Cox et al., 2000). Human immune system is vulnerable to the stress generated by the production of cellular reactive oxygen species (ROS) (Devasagayam & Sainis, 2002). The antioxidants neutralize the harmful ROS and protect the body immune system from oxidative stress, DNA damage from free radicals (De la Fuente & Victor, 2000; Feig, Reid, & Loeb, 1994; Guyton & Kensler, 1993).

Vitamin C (Ascorbic acid) is the most important vitamin in fruits and vegetables. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables. Most of the plant and animal species have the ability to synthesize vitamin C (*L*-ascorbate) from glucose and galactose through uronic acid pathway but man and other primates cannot do so because of deficiency of enzyme gulonolactone oxidase (EC 1.1.3.8) required for its biosynthesis (Nishikimi *et al*., 1994). Vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic function of the body (Jaffe, 1984). It reacts with singlet oxygen and other free radicals and works as an antioxidant that protects body from free radical damage (Sarkar *et al*., 2009). It has significance and beneficial effect respect to human disease such as arteriosclerosis, cardiovascular diseases, diabetes, neurodegenerative disease and cancer (Rekha *et al*., 2012; Choi *et al*., 2007). Adequate intake of vitamin C is effective in lowering the risk of developing cancers of the breast, cervix, colon, rectum, lung, mouth, prostate and stomach (Chambial *et al*., 2013). It lowers blood pressure and facilitates the conversion of cholesterol into bile acids and hence lowers blood cholesterol levels (Rath, 1993). The key role of AA is known to enhance the availability and absorption of dietary nonheme iron in the gut by reducing insoluble and unabsorbable ferric to ferrous state (Zhang *et al*., 2013). It's supplementation is found to facilitate the dietary absorption of iron. The human body cannot produce ascorbic acid, that`s why it must be obtained entirely through diet. Requirement of daily allowance (RDA) of vitamin C for children 20mg, for adult 30 mg and for pregnant and lactating mother 50 mg (Ali *et al*. 2015). A very small daily intake of vitamin C (10-15 mg/day for an adult) is required to avoid deficiency and stave off scurvey (Kallner, 1986).

Lycopene is the red carotenoid pigment found predominantly in fruits and vegetables, most especially in tomato species (Basu and Imrhan, 2007). Lycopene was reported to be a highly effective antioxidant owing to its ability to act as a free radical scavenger and has the highest singlet oxygen quenching rate of all the carotenoids tested from biological systems (Rao and Rao, 2007). Lycopene is recognized to be one of the greatest effective antioxidant between nutritional carotenoids. According to Etminam et al., (2004), daily intake of one or more serving of tomato or lycopene content other vegetable may play a role in the prevention of prostate cancer. Giovannucci et al., (2002) also suggests that increased consumption of tomato based products (especially cooked tomato products) and other lycopene

containing foods may reduce the occurrence of prostate cancer. This indicates that adequate intake of tomatoes and other vegetables may help reduce the incidence of prostate cancer in the community.

For better utilization of vegetables as a human food, clear understanding of their nutrition value as well as the content of vitamin C and lycopene estimation is essential. A variety of leafy and fruits vegetables are available in tropical Bangladesh. The amount of ascorbic acid and lycopene in plants varies greatly, depending on such factors as the variety, weather and maturity (Chaney *et al*., 1979). The objective of this work is to determine the vitamin C and lycopene content in some commonly consumed winter vegetables in Noakhali, Bangladesh. The results were compared with previously published results by similar and different techniques.

I. Material and Methods

The investigation was carried out in the laboratory of the division of post-harvest, 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.

2.1. Plant Materials

The experiment was conducted in an open field. During its growing season all standard growing measures have been applied. The experiment was consisted of sixteen winter vegetables species. From these three were root vegetables like beet root, carrot and red radish; four were fruit vegetable roselle fruit, cherry tomato, red tomato and black tomato (Table 1) and 10 were leafy vegetables like roselle leaf, garden purslan, cabbage, sorrel, beet spinach, water cress, red radish leaf, hydrocotyle, goose foot and goat weed (Table 2). Samples for the study were collected from research field of regional station, Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN), Noakhali, Bangladesh. For the purposes of this research, samples were harvested at full stage. All the samples were thoroughly cleaned using deionised water to remove adhering contaminants. The vegetables were packed with 0.5mm polythene bag and transferred to the lab for nutritional analysis.

2.2. Vitamin C determination Procedure

The ascorbic acid (AA) content was determined as per the procedure prescribed by Pleshkov (1976). Twenty (20) g vegetable sample was taken in a warring blender. The sample was homogenized with warring blender by adding 50 mL distilled water. The homogenized solution was transferred into a 100 mL volumetric flask and made it up to the mark with distilled water and then centrifuged. The supernatant liquid was again collected in the 100 mL volumetric flask. This was the extract solution for the determination of ascorbic acid. For estimating free ascorbic acid 10 mL of prepared extract was taken in conical flask. Five mL 5% KI, 2 mL of 2% starch solution, 2 mL glacial acetic acid was added to the extract. Finally it was titrated with 0.001N KIO_3 solution. Free ascorbic acid was quantified by using the following formula:

$$\text{Vitamin C (mg/100g)} = \frac{f.V_1.V_2.100}{W.V_3}$$

Where,
f= 0.088 mg vit. C for 1 ml of 0.001N-KIO_3
v_1= titrated vol. of 0.001N-KIO_3 (ml)
v_2= total vol. of the sample (ml)
v_3= vol. of sample titrated with 0.001N-KIO_3 (ml)
w= wt. of the plant sample taken (g)

2.3. Lycopene determination Procedure

One day before prepared a mixture solvent Acetone:Hexone (2:3) by using 40 mL Acetone and 60 mL hexane to 100 mL mixture solvent and then placed solvent in the refrigerator. The sample was homogenized with warring blender. Taken 10g homogenized samples tissue in a 50 mL graduated orange cap tubes. Then added 16 mL of cold acetone-hexane mixture and placed the caps tightly. Vertexing the tubes for 60 seconds and then placed in the freezer (-300C). Samples were then left at room temperature for 5 minutes to allow for the separation of both phases i.e. the polar and non-polar phases. Placed the supernatant (only hexane) in other tube (extraction pool), using a plastic pauster pipette and put the cap. The absorbance of the filterate was measured at 663nm, 645nm, 505nm and 453nm by spectrophotometer at the same time.

Lycopene content was determined as per the procedure prescribed by Nagata *et al*., 1992. Calculation of lycopene was performed by using following formula (mg/100g) on fresh wt. basis.

Lycopene = 0.204[**645**]+0.372[**505**]-0.045[**663**]-0.0806[**453**]

where bold figures indicate optical density.

2.4. Statistical analysis

The mean and standard deviation (SD) of vitamin C and lycopene for the samples were performed using the computer software Microsoft Excel.

Table 1. List of root and fruit vegetable selected for vitamin C and lycopene 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	Leaf, root
04	Roselle fruit	Fruit Vegetable	*Hibiscus sabdariffa*	Malvaceae	Leaf, fruit
05	Cherry tomato		*Solanum lycopersicum* var. *cerasiforme*	Solanaceae	Fruit
06	Red tomato		*Solanum lycopersicum*	Solanaceae	Fruit
07	Black tomato		*Solanum lycopersicum*	Solanaceae	Fruit

Table 2. List of leafy vegetables selected for vitamin C and lycopene determination

Sl No	English Name	Scientific Name	Family	Parts use
01	Roselle leaf	*Hibiscus sabdariffa*	Malvaceae	Leaf, fruit
02	Garden purslan	*Portulaca oleracea*	Portulacaceae	whole plant
03	Cabbage	*Brassica oleracea var capitata*	*Cruciferae*	whole plant

04	Red radish leaf	*Raphanus sativus*	Cruciferae	Leaf, root
05	Sorrel	*Rumex vasicarious*	Polygonaceae	whole plant
06	Spinach	*Spinacea oleracea*	Amaranthaceae	whole plant
07	Hydrocotyle	*Hydrocotyle asiatica*	Mackinlayaceae	whole plant
08	Goose foot	*Chenopodium album*	Chenopodiaceae	Leaf
09	Water cress	*Enhydra fluctuans*	Asteraceae	whole plant
10	Goat weed	*Blumea lacera*	Asteraceae	Leaf

II. Results and Discussions

Variation in ascorbic acid among the winter vegetable genotypes ranged between 7.13±0.907 and 19.71±1.429 mg/100g. The result showed sorrel has maximum vitamin C content and red radish had low vitamin C content. This findings are similar as the previous study of Isabella et al.,2010. Sorrel is the non-conventional leafy vegetable. It is not available in local markets and but it is not expensive, the considerable amount of vitamin C presents in these vegetables showed that when they are consumed in relative large amount, they will certainly contribute to the daily human dietary intake of the vitamin. It is to be noted that the requirement of vitamin C increases during pregnancy, lactation, adolescence, hyperthyroidism, infection and after surgery (Hassan and hassan, 2008). Maintenance of daily dietary intake of vitamin C leads to the prevention of scurvy which is the deficiency disease state of vitamin C. This disease state has been shown to be high in children and the elderly (Chambial *et. al*, 2013; Kumar *et al*., 2013).

In the present investigation, lycopene content ranges from 13.45±4.04 to 6648.34±9.89 µg/100g. The findings were in accordance with Burns *et al*., 2003, who reported lycopene content of 522.5µg/g. Mladenovic *et al*., 2014 also reported similar results (0.031mg /100g-4.330mg/100g). Lycopene is known to be one of the most potent antioxidants among dietary carotenoids (Argarwal and Rao, 2000). Knowing the lycopene content of commonly consumed foods is useful in planning dietary strategies to satisfy the reference daily intake for antioxidant and also as useful indices of potential health benefits, of individual plant-based foods. The beneficial effects of fruits and vegetables are hypothesized, owing at least to their antioxidant's contents (Collins, 1999).

The result presented in the study has shown that lycopene content varied widely among vegetables indicating the need of different servings of these foods. Among the food samples analyzed, tomato had the highest content of lycopene. Lycopene content in the tomato commonly consumed in Bangladesh was high in vegetables. This observation is similar to the reports of Grossman *et al*., (2004) which indicates that tomatoes and tomato-based sauces, juice and ketchup account for more than 85% of the dietary intake of lycopene of most people who include them in their diet. Lycopene content in the tomato-based product was higher than in fresh tomato which is likely due to the higher moisture content of fresh tomatoes than the processed tomatoes paste. Khan *et al*., (2008) also reported that, lycopene in tomato pastes was four times more bioavailable than in fresh tomato. According to report of Khan *et al*., (2008), cooking and crushing of tomatoes (as in canning process) and with addition of serving oil, greatly increases the assimilation of lycopene from the digestive tract, into the bloodstream.

3.1. Roots and fruits vegetables

A result presented in Table 3 for determination of vitamin C and lycopene content in selected roots and fruits vegetable. The highest content of vitamin C revealed in black tomato (27.48±1.45 mg/100g) which was followed by cherry tomato (26.04±1.12), red tomato (21.36±0.954), roselle fruit (16.25±0.874 mg/100g) and carrot (11.58±0.622 mg/100g). Researchers found vitamin C content in roselle fruit was 58.30 ± 2.4 mg/100g by Zablul Tareq *et al*., 2021. Kumar *et al*., 2013 analyzed the vitamin C of carrot and found 29.92±0.04 mg/100g. Other researchers also analyzed vitamin C of carrot and found 2.6 ± 0.72 mg/100g (Deekshika *et al*., 2015). The lowest vitamin C content was observed in red radish (7.13±0.907 mg/100g) which was almost similar to beet root (7.20±0.490 mg/100g) (Table 3). Sharaa and Mussa (2019) analyzed the vitamin C of red radish and found more amount 61.54±0.06 mg/100g. The vitamin C content in beet extract was 54.99±0.06 mg/100g found by Lembong *et al*., 2019. Lycopene varied in roots and fruits vegetables ranging from 25.25±3.08 µg/100g in beet root to 673.98±5.76 µg/100g in cherry tomato. Beetroot had the lowest lycopene content was 0.33 - 0.5% that reported by Lilwani and Nair, 2015. High lycopene content value was 13.65 to 43.60% in red tomato found by Kunnumakkara, 2014. Lycopene is fat soluble thus oil helps its absorption (Khan, *et. al.*, 2008). This indicates that individual should include a lot of tomato, especially tomato paste in combination with some edible oil in his/her meals. Regular intake of fruits and vegetables, with inclusion of tomato-based products, may result in regular intake of 20mg lycopene or more /day (EPSA, 2005).

Table 3: Vitamin C and lycopene content of selected root and fruit vegetables

Sl No	English Name	Vitamin C (mg/100g edible portion)	Lycopene (µg/100g edible portion)
01	Beet root	7.20±0.490	25.25±3.08
02	Carrot	11.58±0.622	65.48±4.33
03	Red Radish	7.13±0.907	49.71±4.71
04	Roselle fruit	16.25±0.874	59.72±6.09
05	Cherry tomato	26.04±1.12	6648.34±9.89
06	Red tomato	21.36±0.954	4107.27±8.13
07	Black tomato	27.48±1.45	1365.21±4.26
	Min.	7.13±0.907	25.25±3.08
	Max.	27.48±1.45	6648.34±9.89

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

3.2. Leafy vegetables

In case of leafy vegetable the highest content of ascorbic acid was recorded in sorrel (19.71±1.429) which was followed by goat weed (17.76±1.264 mg/100g), beet spinach (17.71±1.532 mg/100g) and roselle leaf (17.36±0.844 mg/100g) (Table 4). Sorrel (*Rumex vesicarius* L.) locally sorrel is an edible green leafy plant that belongs to the family of Polygonaceae. This shrub grows annually during winter season in Bangladesh. This is content of high dietary antioxidant (vitamin C). The intake of dietary

antioxidant (vitamin C) leads to protection against non-communicable diseases i.e. cancer, cardiovascular diseases and cataract (Ghafar *et al*. 2010; Imran *et al*. 2011). Rahman *et al*., 2007 and Deekshika *et al*., 2015 analyzed vitamin C content of beet spinach and revealed 21.439±0.153 mg/100g and 26.31 ± 0.98 mg/100g, respectively. Researchers found vitamin C content in roselle leaves was 94.88 ± 1.0 mg/100g by Zablul Tareq *et al*., 2021. The lowest vitamin C content of selected leafy vegetable was found in radish leaf (11.40±1.664 mg/100g) which was followed and similar with cabbage (11.92±1.287 mg/100g) (Table 4). Kumar *et al*., 2013 analyzed vitamin C content of Cabbage and found 35.55±0.09 mg/100g. It is observed that three leafy vegetable like roselle leaf (17.36±0.844 mg/100g), spinach (17.71±1.532 mg/100g) and hydrocotyle (17.76±1.264 mg/100g) have similar vitamin C content.

Table 4: Vitamin C and lycopene content of selected leafy vegetables

Sl No	English Name	Vitamin C (mg/100g edible portion)	Lycopene ((µg/100g edible portion)
01	Roselle leaf	17.36±0.844	673.98±5.76
02	Garden purslan	15.44±0.791	211.02±5.12
03	Cabbage	11.92±1.287	208.85±2.79
04	Sorrel	19.71±1.429	352.29±3.89
05	Spinach	17.71±1.532	474.19±4.63
06	Water Cress	14.03±1.313	138.98±4.95
07	Radish leaf	11.40±1.664	15.85±2.03
08	Hydrocotyle	17.76±1.264	13.45±4.04
09	Goosefoot	16.96±1.096	441.08±4.85
10	Goat weed	13.50±1.112	551.18±6.02
	Min.	11.40±1.664	13.45±4.04
	Max.	19.71±1.429	673.98±5.76

Table 5: Comparison of the vitamin C content (mg/100g sample) in some winter vegetables samples between the current study and previous studies

Samples	Previous study			Present study (mg/100g)
	Vitamin C (mg/100g)	Methods	Reference	
Tomato	26.73±0.52	Titration Method	Elhefian et al., 2019	Red tomato 21.36±0.954 Black tomato 27.48±1.45
Beet extract	54.99±0.06	Titration Method	Lembong *et al.*, 2019	7.20±0.490
Carrot	29.92±0.04	Volumetric method	Kumar *et al.*, 2013	11.58±0.622
	2.6 ± 0.72	Volumetric method	Deekshika *et al.*, 2015	
Red Radish	61.54±0.06	Titration Method	Sharaa and Mussa, 2019	7.13±0.907
Roselle fruit	58.30 ± 2.4	Volumetric method	Zablul Tareq *et al.*, 2021	16.25±0.874
Roselle leaf	94.88 ± 1.0	Volumetric method	Zablul Tareq *et al.*, 2021	17.36±0.844
Cabbage	35.55±0.09	Volumetric method	Kumar *et al.*, 2013	11.92±1.287
Spinach	21.439±0.153	UV-spectrophotometric method	Rahman *et al.*, 2007	17.71±1.532
	26.31 ± 0.98	Volumetric method	Deekshika *et al.*, 2015	

These findings conclusively suggest that the locally available leafy vegetables are good source of vitamin C. Vitamin C is important to human health, and many species need a dietary source to stay healthy. The locally available vegetables which was analyzed contain relatively good amount and a good source of vitamin C.

Lycopene varied in leafy vegetables ranging from 13.45±4.04 µg/100g in hydrocotyle to 673.98±5.76 µg/100g in roselle leaf. Among the leafy vegetables, roselle leaf had the highest lycopene content followed by goat weed (551.18±6.02 µg/100g), spinach, goosefoot while hydrocotyle had the lowest lycopene content.

Table 6: Comparison of the lycopene content (mg/100g sample) in some winter vegetables samples between the current study and previous studies

Samples	Previous study			Present study (mg/100g)
	Lycopene (mg/100g)	Methods	Reference	
Fresh tomato	4.59±0.98	Spectrophotometer method	Fadupin *et al.*, 2012	Red fresh tomato 4.107±8.13
Tomato paste	6.82±1.44			
Beet	0.57±0.14	Spectrophotometer method	Jayamaha et al., 2019	0.025±3.08
Roselle fruit	0.164±0.04	HPLC	Wong *et al.*, 2002	0.059±6.09
Cabbage	1.35±0.86	Spectrophotometer method	Jayamaha et al., 2019	0.208±2.79

Within winter vegetables fresh cherry tomato had the highest concentration of lycopene content. The value obtained is also within the range reported by Fadupin et al., (2012) and Khachick et al., (1992).

IV. Conclusion

Vegetables provide vital nutrients required for human health and wellbeing. This study has shown that cherry tomato, red tomato, red tomato and roselle fruit are very rich source of vitamin C and lycopene under the roots and fruits vegetables. Roselle leaf, sorrel, spinach and goosefoot are high in vitamin C and lycopene content compared to other leafy vegetables.The results suggest that the vegetables if consumed in sufficient amount would contribute greatly towards meeting the nutritional requirement for normal growth and also could provide adequate protection against diseases arising from malnutrition. In rural areas, winter vegetables play important role as nutritional source. Winter vegetables are usually considered as the cheapest source of food for vitamins a, micronutrients and antioxidant supplementation to combat nutrients deficiencies. Ascorbic acid is necessary for healthy teeth, gums and bones and is essential for proper functioning of adrenal and thyroid glands. Lycopene fight against cancer in our body. They are antioxidants and as such act as a general de–toxicant. The locally selected winter vegetables can be consumed to meet the daily requirement of vitamin C and lycopene.

Acknowledgement

The authors are highly thankful to the Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN),

Arayhazar, Narayangonj for providing technical and financial assistance during the research programed.

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