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REGULAR ARTICLE

Chemical composition, Antioxidant capacities and storage stability of *Citrus macroptera* and *Garcinia pedunculata* fruits

M. Z. Islam[1,2*], M. M. Hoque[1], S. M. Asif-Ul-Alam[1] and K. Monalisa[1]

[1]*Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet, Bangladesh*
[2]*Department of Bioindustrial Sciences, Graduate School of life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan*

Abstract

An attempt was made to gain an insight about chemical compositions and antioxidant capacities of selected underutilized fruits Satkara (*Citrus macroptera*) and Taikor (*Garcinia pedunculata*) in Sylhet, Bangladesh. The analysis showed β Carotene and Vitamin C in Satkara 22±1.50 mg/100g and 210.43±0.40 mg/100g whereas Taikor contains 45±1.00 and 142.83±2.03 mg/100g respectively. The mineral composition of both fruits is appreciable. Total polyphenol content (TPC) and total flavonoids (TF) in Satkara and Taikor were 22.76±0.52 mg GAE/100g and 18.98±0.65 mg GAE/100g, 23.38±1.0 mg RE/g and 18.33±1.12 mg RE/g respectively. The antioxidant capacities of these fruits were evaluated by DPPH and ABTS method, values expressed as TEAC (Trolox Equivalent Antioxidant Capacity). In case of ABTS showed that the value 19.06±0.18 TEAC (μM/g) and 2318.44±5.60 TEAC (μM/g); for DPPH showed 16.20±0.04 TEAC (μM/g) and 1813.88±12.62 TEAC (μM/g) in Satkara and Taikor respectively. The total polyphenol content showed the positive correlation with the antioxidant capacities and ascorbic acid contents of these fruits. Storage studies at two different temperatures at 5ºC for 15 days and -18ºC for 3 months long storage period antioxidant activity shown to be stable but vitamin C content decreases significantly. The results demonstrate that *Citrus macroptera* and *Garcinia pedunculata* fruits contained the considerable levels of nutrients and antioxidant capacity.

Key words: Satkara, Taikor, Chemical composition, Antioxidant capacity, Storage study

Introduction

Fruits especially citrus are the important sources of major groups of phytochemicals that have been suggested as a natural source of antioxidants. The major groups of antioxidants presents in citrus are polyphenols (e.g. flavonoids) and vitamins such as vitamin C and E. Against the anti-inflammatory, anti cancer, anti tumor and blood clotting, citrus fruit extracts demonstrate the positive effect on human (Du and Chen, 2010). Despite many reports on commonly consumed fruits of their phenolic content (PC), nutritional value and antioxidant capacity (AC), a very few reviews are available for *Citrus macroptera* and *Garcinia pedunculata* fruits. The underutilized fruits found to be the potential sources of phytochemicals or even unique health promoting compounds. Recent year the interests in research and development activities has arisen in exploiting on underutilized fruit species because of their positive role against the diseases and enhancing human well-being daily life (Schreckinger et al., 2010). The present study shows interests in the two important underutilized fruits specially grown in Bangladesh, namely *Citrus macroptera* Montr. (family-Rutaceae) (*C. macroptera*) commonly called Satkara (wild orange) and Taikor (*Garcinia pedunculata*).

Satkara (*Citrus macroptera*) fruits grown on tree, which has thorns about 5m in height. The fruit is about 6-7 cm in diameter and the skin has a fairly smooth. The ripe fruit turns yellow, but the pulp is greenish yellow. It produces very little amount of juice with very sour and bitter in taste (Nizamuddin et al., 2014). According to Grover et al. (2002) traditionally Satkara has good reputations to remedies abdominal pains, hypertension, in flu, fever and diarrhea in infants. The matured fruit is

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*Corresponding Author

M. Z. Islam
Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh

Email: zohurulislam.engg@gmail.com; zohurul-fet@sust.edu

used in cooking for flavoring curry, pulses and vegetables and also for pickle preparation. Taikor (*Garcinia pedunculata*) the tree has a fluted trunk with short spreading branches. The roundish fruit of a diameter ranging between 8 to 12 cm and matured fruit is greenish yellow. Some prominent Garciniaspecies are known to have good medicinal value and fruit extract has traditionally very well known for treatments of various diseases (Deore et al., 2011). The mature fruit is eaten cooked or raw and also for pickle preparation. *Citrus macroptera* and *Garcinia pedunculata* fruits selected for further investigation into the stability of antioxidant capacities and chemical compositions as well as the vitamin C content during storage of two different temperatures at 5°C and -18°C for three month long storage period. To the best of our concern, no review has found on the effect of storage conditions to evaluate the stability of nutritional and functional aspects of these fruits. Only a few research has conducted on the total polyphenol and antioxidant activity of Taikor (*Garcinia pedunculata*) fruit and no studies have been published about the food functional, antioxidant and phenolic compositions of Satkara (*Citrus macroptera*).

Thus, the present study evaluates two types of underutilized fruits namely: Taikor (*Garcinia pedunculata*) and Satkara (*Citrus macroptera*) as new potential natural sources of nutrients, phenolic compounds as well as good sources of antioxidants.

Materials and Methods

Samplesand Preparation of extracts

The fresh Satkara (*Citrus macroptera*) and Taikar (*Gracinia pedunculata*) fruits were collected from the Citrus Research Station, Sylhet, Bangladesh. The whole fruits were cleaned and washed with distilled water. Edible portion of fruits was separated carefully. Fruits were homogenized and extracted with 200ml of ethanol: acetone (7:3 v/v) for 1 h at 37ºC according to Lee and Wicker (1991). The extract was filtered through Whatman No.41 paper and rinsed with 50ml of ethanol: acetone (7:3 v/v). Extraction of the residue was repeated using the same conditions. The two filtrates were combined and then stored at −20◦C until used for analysis of the total polyphenol, total flavonoids and antioxidant capacity.

Figure 1. Satkara (*Citrus macroptera*) fruits and its internal part.

Figure 2. Taikor (*Garcinia pedunculata*) fruits and its internal part.

Physicochemical analysis

The edible portion of these fruits was analyzed for moisture by moisture analyzer (IR-60 Denver Instrument), protein content (Micro Biuret method), total soluble solid (Hand Refractometer, ERMA 58-92) and fat, fiber, pH contents with the following standard methods as described by AOAC (2004). The concentration of mineral and heavy metal elements were determined using Atomic Absorption Spectrophotometer (Thermo-scientific iCE 3000).

Thiamine and Riboflavin were estimated by the methods of Coward (1938); Gyorgy and Pearson (1967). The Ascorbic acid (AA) content was determined using the 2, 6-dichlorophenol-indophenol. It was used L-ascorbic acid to prepare a standard solution (0.5 mg/ml) and the concentration was calculated by comparison to the standard and expressed as mg/100 g fresh mass.β carotene content was estimated by the method of Holden, 1981 and values were expressed as mg/100g.

Determination of total polyphenol content (TPC) and total flavonoids (TF)

The amount of total polyphenols was determined according to the Folin–Ciocalteu Spectrophotometric method (Rapisarda et al., 1999). Absorbance was measured at 765 nm. Total polyphenols was expressed as Gallic acid equivalents (mg/100g of GAE). Gallic acid (wako-071-06095) standard solutions were prepared at a concentration ranging from 0 to 1000mgL^{-1}.

Total flavonoid content was determined using colourimetric method described by Abu Bakar et al. (2009), as adapted from Dewanto, Wu, Adom, and Liu (2002). Briefly, 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube followed by the addition of 0.15 ml of 5% $NaNO_2$solution. After 6 min, 0.3 ml of a 10% $AlCl_3.6H_20$ solution was added and allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was mixed well by vortexing. The absorbance was measured immediately at 510 nm using a spectrophotometer. Results were expressed as mg rutin (wako-181-00341) equivalents in 1 g of sample (mg RE/g)

Measurement of antioxidant capacity

The antioxidant capacity was determined by the modified free radical DPPH method (Brand-Williams et al., 1995), based on free radical scavenging by antioxidants, and the ABTS method (Re et al., 1999), where the free radical is generated by a chemical reaction with potassium persulfate, was used to determine the antioxidant activity with modifications.The extract was obtained from about 10 g of sample in 40 ml 50% aqueous methanol and 40 ml 70% aqueous acetone centrifuged twice at 15,000 × g for 15 min (Hitachi, model Himac CR21E centrifuge, Tokyo, Japan), and three dilutions prepared using the supernatant (1:5, 1:10 and 1:15). This procedure was adapted from Larrauri et al. (1997).

For the DPPH method, a 100 µL aliquot of each dilution was added to 3.9 ml of DPPH radical (Wako, Japan), and the reading made at 515 nm in an Ultrospec 3100 pro spectrophotometer (HITACHI U-1900 Spectrophotometer) after 30 min, using methanol as the blank. For the ABTS method, an aliquot of 30 µL of each dilution was added to 3.0 ml of ABTS radical (Wako), and the reading made in the spectrophotometer at 734 nm after 6 min of reaction, using ethanol as the blank and a standard curve prepared using Trolox (Wako, Japan). The analyses of the extract were carried out in triplicate and the results presented in µM of Trolox equivalents (TE)/g.

Statistical analysis

All samples were prepared and analyzed in triplicate. Statistical analysis was done by one-way analysis of variance. The Pearson correlation coefficient (R) and p-value were used to show correlations and their significance (SAS 9.0 for Windows). Probability values of $p < 0.05$ were considered statistically significant.

Results and Discussion

Proximate analysis

Proximate analysis represents the necessary information related to the identification, classifications and as well as to express the nutritional quality and value of the food materials. The proximate analyses of Satkara and Taikar are presented in Table 1. Table exhibits moisture content in Satkara 90.40±2.0% and 88.2±2.0% in Taikar. Due to the high value of the moisture percentage, water soluble enzymes and co-enzymes show greater response and which are responsible for the metabolic activities. From proximate composition, the high amount of fiber and total soluble solids can be noticed for both Satkara and Taikar.

For maintaining good and sound health, a minute amount of vitamin should be taken in the daily meal. The common and easy sources of vitamins are fresh fruits. The β-Cryptoxanthin, β-

carotene and α-carotene are the primary sources of pro-vitamin A, those components are derived from citrus. Table 2 shows that Satkara contain 22±1.5mg/100g and Taikar 45±1 mg/100g ofβ Carotene respectively. It is found that β Carotene prohibits the free radical damage in lipid membrane in under low oxygen partial pressure. In the absence or in shortage of vitamin C and vitamin E, β Carotene might act as a protective antioxidant and it will become more effective at high oxygen pressure. Table 1 show that both Satkara and Taikor contain small amounts of Thiamine (0.08±0.01mg/100g; 0.03±0.01mg/100g) and Riboflavin (0.01±0mg/100g; 0.02±0mg/100g).

Citrus fruits are the principal source of Vitamin C which is an important water-soluble antioxidant. Vitamin C plays vital role to prevent the oxidative damage of biomolecules and terminates reactive oxygen and nitrogen species. Table 1 represents Vitamin-C content in Satkara 210.43±0.40 mg/100g and 142.83±2.03 mg/100g. Taikor (*Garcinia pedunculata*) and Satkara (*Citrus macroptera*) fruits contained high amounts of Vitamin C. The RDA (Recommended Dietary Allowance) value for vitamin C is 60 mg per day. Daily intake of 60 mg of vitamin C would prevent the development of scurvy for about one month in a diet lacking vitamin C.

Table 1 shows Satkara contains 25±1.0 mg and Taikar 18±0.75 mg of Ca. Calcium and Phosphorus are required in a proper amount for the body growth and maintenance. Above table indicates that Satkara contains 10±0.75 mg and Taikar 17±0.25mg of Phosphorus. For the development of bones Phosphorus is an essential element. Due to the lacking of phosphorus- calcium balance, diseases like osteoporosis, arthritis, pyorrhea, rickets and tooth decay occurred. Magnesium (Mg) contents in Satkara 10±0.75 mg and in Taikar 23±1.0 mg. The RDA (Recommended Daily Allowance) value of Magnesium is 400mg/day for men 19-30 years old and 310 mg/day for women 19-39 years old (FNB, 1997) which are far too high than the level obtained in this study. Trowbridge and Martorell (2002), stated that more than one billion people are affected by anemia, which is caused by the iron deficiency. Iron deficiency is also responsible for the reduction of work ability, damage of behavior and rational appearance and reduced immunity of the body (Dioxin et al., 2004). The table represents the both Satkara and Taikor contains very small amounts of Iron (Fe). In the regulations of plasma volume, nerve and muscle contraction, maintaining balanced in acid-base, Sodium and Potassium shows the important role (Akpanyung, 2005). Table 1 shows Satkar and Taikor contains 3.5±0.01mg; 1.8±0.01mg of sodium respectively. The potassium content is high in Taikar 106±2.0 mg and 89±1.0 mg in Satkara. Both Satkara and Taikor contain a small amount of copper. Zinc is a necessary element for human growth and it increases resistance to infection (Black, 2003). About 20% people worldwide are at a risk of zinc deficiency (Hotz & Brown, 2004). Satkara contain 0.21±0.01mg and Taikor 0.15±0.01mg of Zn. The present study shows that Zinc content in Shatkara and Taikar is appreciable.

Table1. Proximate composition of *Citrus macroptera* and *Garcinia pedunculata* fruits (mg/100g).

Parameters	Satkara	Taikar
Moisture (%)	90.40±2.0	88.2±2.0
Protein (%)	0.40±0.7	0.50±0.40
β Carotene	22±1.50	45±1.00
Thiamine	0.08±0.01	0.03±0.01
Riboflavin	0.01±0	0.02±0.00
Ascorbic acid	210.43±0.40	142.83±2.03
Calcium (Ca)	25±1.0	18±0.75
Phosphorus (P)	22±0.05	17±0.25
Magnesium (Mg)	10±0.75	23±1.00
Iron (Fe)	0.15±0.02	0.08±0.01
Sodium (Na)	3.5±0.01	1.8±0.01
Potassium (K)	89±1.0	106±2.00
Copper (Cu)	0.07±0.01	0.12±0.01
Zinc (Zn)	0.21±0.01	0.15±0.01
Calcium (Ca)	25±1.0	18±0.75

Mean value ± standard deviation; n = 3

Bioactive compounds and antioxidant capacity

According to (Liu, 2004), Phenolic compounds are mostly composed of flavonoids, phenolic acids, stilbenes, coumarins and tannins. They perform as a free radical scavenger (Shahidi et al., 1992) and mostly include flavonoids, phenolic acids, stilbenes, coumarins and tannins (Liu, 2004). The present studies explain that Satkara contains the TPC of 22.76±0.52 mg GAE /100g and Taikor 18.98±.65 mg GAE /100g as shown in Table 2. Our study shows that Satkara contain 23.38±1.0 mg RE/g and Taikor 18.33±1.12 mg RE/g of TF.

The chemical structure of the components, their synergistic interaction and specific conditions for various assays may affect the relationship between the antioxidant activity and phenolic compounds of those components (Huang et al., 2005).

Two well-known free radical scavenging method ABTS and DPPH were used in this study. In this method free radicals involved in the color disappearances and converted to a colorless compound. The amount of scavenged ABTS and DPPH represent the degree of discolorations. Table 2

illustrates the antioxidant activity of Satkara and Taikar estimated by ABTS and DPPH method having an enormous quantity of antioxidant activity. Pearson correlation coefficient showed that there is a positive relation among TP, Vit-C, ABTS, DPPH in Satkara and Taikor, except moderate negative (-0.461) relation between DPPH and ABTS in Taikor (Table 3). Similar studies on the antioxidant activity of 18 non-traditional, tropical fruits in Brazil had showed the negative correlation between ABTS and DPPH (Rufino, 2010). Although Satkara showed highest positive (0.915) correlation between TP and DPPH but Taikor showed very weak positive correlation. This is because of the presence of high amount of vitamin C, carotenoid and minerals, those has the reducing property (Deepa et al., 2006). Jagdish et al. (2007) stated another reason that may occur due to the agronomic and environmental influences. The methodological limitations also may affect the total antioxidant determinations (Kaur and Kapoor, 2001). In Taikor Vitamin-C and Total Polyphenol exhibited highest positive (0.894) correlation.

Storage stability studies of ascorbic acid, Total polyphenol and antioxidant capacity

Water activity, unlike moisture content, can determine shelf stability. It can predict which microorganisms will be potential sources of spoilage and infection and also chemical stability. The inspection at 5ºC for 15 days shows the gradual decrease of moisture content. Moisture content changes significantly at 5, 10, 15 days correspond to o day and changes is insignificant ($p < 0.05$) among 5to 15 days in Satakar. In Taikor moisture content significantly changes with increasing the time. The initial moisture content was 88.53±0.15 which subsequently reduces to 83.33±0.17at 15 days of storage. Vitamin C content in Shatakar and Taikar has decreased with increasing time, but in case of Taikar it shows insignificant changes from 0 to 5 days at 5°C. The length of the storage time and the temperature may affect the degradation process (Gordon and Samaniego- Esguerra, 1990).

The overall antioxidant activity is the results of the interactions among the various food components such as vitamin C, carotenoid, vitamin E, phenolic compounds. It is really difficult to predict the individual effects of the component on the antioxidant capacity (Pinelo et al., 2004). The fruits were proposed to refrigeration temperature; Satakara shows significant decreases in activity the ABTS method, but showed an insignificant increase on the 10th day and by DPPH method activity increases day by day. This result demonstrated that Satkara capable of maintaining its bioactive properties stable during the first 15 days. This oscillation in activity is in line with the blueberry juice study by Piljac-Zegarac et al. (2009). In case of tailor activity decreases with increasing day, but at 10th day it is observed slight, insignificant increase of activity by ABTS method. By DPPH method activity increases with storage time, but 5th to 10th the changes were insignificant. Thus result can be concluded that the low temperature storage of Satakar and Taikor is capable of maintaining its bioactive properties stable during the first 15 days.

Table 2. Bioactive compound and antioxidant activity in *Citrus macroptera* and *Garcinia pedunculata* fruits.

Elements	Satkara	Taikar
Total Polyphenol (mg GAE/100g)	23.17±1.57	19.45±1.0
Total Flavonoids (mg RE/g)	23.38±1.0	18.33±1.12
Antioxidant activity		
ABTS (μM TE/g fruit)	19.06±0.12	16.20±0.04
DPPH (μM TE/g fruit)	2318.44±5.60	1813.88±12.62

Mean value ± standard deviation; n = 3

Table 3. Pearson's correlation coefficients (R) of Total Polyphenol, Vitamin C contents and Antioxidant capacity of *Citrus macroptera* and *Garcinia pedunculata* fruits.

Parameters	Correlation coefficient (R)		
	ABTS	DPPH	Total Polyphenol
Satkara (*Citrus macroptera*)			
Total Polyphenol	0.429	0.915	
Vitamin-C	0.224	0.806	0.429
DPPH	0.757		
Taikor (*Garcinia pedunculata*)			
Total Polyphenol	0.789	0.181	
Vitamin-C	0.431	0.602	0.894
DPPH	-0.461		

Table 4. Stability of moisture, vitamin C and antioxidant activity of the whole Satkara (*Citrus macroptera*) and Taikor (*Gracinia pedunculata*) fruit stored under refrigeration (5ºC) for 15 d.

Satkara (*Citrus macroptera)*				
Time Day	Moisture %	Vitamin-C (mg/100g)	ABTS (µM TE/g fruit)	DPPH (µMTE/g fruit)
0	90.13±0.25^{a}	210.43±0.40^{a}	19.06±0.12^{a}	2318.44±5.60^{a}
5	89.30±0.26^{b}	206.32±1.15^{b}	17.76±0.38^{b}	2441.33±37.6^{b}
10	89.08±0.11^{b}	193.78±1.21^{c}	19.06±0.12^{a}	2647.63±50.1^{c}
15	88.80±0.26^{b}	175.25±0.44^{d}	16.02±0.27^{c}	2833.62±37.3^{d}
Taikor (*Gracinia pedunculata*)				
0	88.53±0.15^{a}	142.83±2.30^{a}	16.20±0.04^{a}	1813.88±12.62^{c}
5	87.96±0.21^{b}	139.78±0.56^{a}	14.04±0.06^{c}	1923.01±36.96^{b}
10	86.20±0.30^{c}	124.08±1.83^{b}	14.98±0.27^{b}	1628.82±10.30^{b}
15	83.33±0.17^{d}	101.44±0.98^{c}	13.23±0.21^{d}	2009.79±2.510^{a}

Abbreviations: TE (Trolox equivalents). The analyses were performed in three replicated samples. Values expressed as the mean ± standard deviation. Means with the same letters in the same column indicate no significant difference ($p < 0.05$) between samples.

During the first 3 months of frozen storage (-18°C) moisture content of Satakara increases from 90.13±0.25to 92.84±0.07; but at 2nd month changes is not significant. In Taikor moisture content changes significantly from o day. There is a drastic change in Vitamin C. At the 3rd month of storage vitamin C in Taikor found 98.18±0.14where as at o day it was 210.43±0.40. The antioxidant capacity of both fruits increased significantly by both methods. The value has fallen at 2nd month, although maintaining a value above that of 0 days. This can be explained by reactions that continue to occur after harvest, forming compounds responsible for this increase (Piljac-Zˇegarac and Sˇamec, 2011); for example, reactions produced via metabolism of the phenolics (Kalt et al., 1999), resulting from cellular disruption caused by thawing the fruit before the time of analysis, improving the extraction of these compounds (De Ancos et al., 2000). In a study by Connor et al. (2002), different blueberry cultivars, mature and not matter, were refrigerated at 5°C for up to 7 weeks. The authors concluded that fruits harvested before complete maturity could be stored under refrigeration for 7 weeks without prejudice to the antioxidant compounds, such as total phenolic compounds and anthocyanin, maintaining their commercial quality. On analyzing the antioxidant behavior of various types of berries and cherries by different methods, during refrigerated storage at 4°C for 30 d, Piljac-Zegarac and Sˇamec (2011) only observed insignificant alterations. Compared with the natural values, the antioxidant capacity of the raspberries was increased significantly Kalt et al. (1999) found no significant alterations in the antioxidant activity of Lowbush and Highbush blueberries as measured by the ORAC method during storage of the fruits for 8 d at 0, 10, 20 and 30°C.

Table 5. Stability of moisture, vitamin C and antioxidant capacity of the whole Satkara (*Citrus macroptera*) and Taikor (*Gracinia pedunculata*) fruit stored under refrigeration (-18ºC) for 90 d.

Satkara (*Citrus macroptera)*				
Time Day	Moisture %	Vit-C (mg/100g)	ABTS (µM TE/g fruit)	DPPH (µM TE/g fruit)
0	90.13±0.25^{c}	210.43±0.40^{a}	14.06±0.05^{d}	2782.97±2.65^{a}
15	92.70±0.26^{a}	192.61±0.54^{b}	17.96±0.02^{bc}	2493.81±6.59^{b}
30	92.18±0.02^{b}	187.39±1.05^{c}	17.48±0.42^{c}	1693.20±12.68^{d}
60	92.42±0.03^{ab}	142.75±0.53^{d}	18.11±0.06^{b}	2453.12±34.2^{b}
90	92.84±0.07^{a}	98.18±0.14^{e}	19.06±0.12^{a}	2318.44±5.60^{c}
Taikor (*Gracinia peniculata*)				
0	88.56±0.15^{d}	142.83±2.03^{a}	14.64±0.41^{c}	2040.96±2.60^{a}
15	88.03±0.01^{e}	135.24±0.02^{b}	16.12±0.10^{b}	2013.67±1.52^{a}
30	89.13±0.02^{c}	120.47±0.03^{c}	16.91±0.03^{a}	1909.66±2.51^{b}
60	89.47±0.025^{b}	105.68±0.01^{d}	15.12±0.03^{c}	1893.49±4.46^{b}
90	90.70±0.040^{a}	100.08±0.03^{e}	15.75±0.04^{b}	1800.33±24.50^{c}

Abbreviations: TE (Trolox equivalents). The analyses were performed in three replicated samples. Values expressed as the mean ± standard deviation. Means with the same letters in the same column indicate no significant difference ($p < 0.05$) between samples.

Conclusion

This study demonstrates that these underutilized fruits of Bangladesh are rich sources of fiber, total phenols, and natural antioxidants with high amount of ascorbic acids. Hence, the use of these fruits may not only be attractive as a potential sources of nutrients, natural antioxidants, could be developed into value added products or medicine, is likely to be economically attractive. The study also shows that both fruits presented good stability with respect to their antioxidant capacity and ascorbic acid content during cold storage, after remaining frozen for a long period and under refrigeration for shorter periods, with little loss of their antioxidant properties. The present research will be beneficial for the consumers and also the cultivars to encourage growing more this fruits, it will be helpful for the nutritionists in estimating the daily intakes of major and minor components presents in this fruit and their impact on health. The study will also be useful for the production of value added product for industrial adoptions.

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Author contributions

S. M. A. U. A. had a great contribution to the review paper. M. M. H. was involved in supervisions. M. Z. I. and K. M. had a major contribution to the overall planning and performing the research successfully.

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