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Research Article

THE EFFECT OF STORAGE ON VITAMIN C STABILITY OF LOCALLY GROWN FRUITS IN BRUNEI DARUSSALAM

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ABSTRACT

Objective: Since humans are incapable of producing Vitamin C, we obtain our daily requirement of Vitamin C from dietary sources such as fruits. It is a common household practice to refrigerate fruits or fruit juices (4°C–5°C). The main aim is to understand the effect of storage and temperature on the Vitamin C level of locally available fruits or fruit juices.

Methods: Four locally grown fruits were selected: Pomelo, papaya, tomato, and watermelon and one imported fruit from the United States of America, i.e., orange to test for Vitamin C stability following refrigeration at 4°C. Vitamin C was measured at day 0, 1, 2, 3, and 7. Determination of Vitamin C was carried out by an accredited Association of Official Agricultural Chemists indophenol method.

Results: At day 0, orange had the highest level of Vitamin C (58.2±0.00 mg/100 ml), while watermelon contained the least (6.7±1.27 mg/100 ml). The Vitamin C in orange and pomelo was stable for at least 24 h, and all fruits experienced continuous loss until the end of the storage period, day 7. Watermelon recorded the highest Vitamin C loss of 82.1%.

Conclusions: Therefore, it is best to consume fresh fruit juice within 24 h, particularly for watermelon to obtain maximal Vitamin C.

Keywords: Fruits, Vitamin C, Storage, Stability.

INTRODUCTION

Vitamin can be defined as an organic compound that is fundamental for normal physiological functions such as maintenance, growth, development, and production [1,2]. It is usually needed in miniscule quantity in order for the body to have optimal functionality. Deficiency or underutilization of certain vitamin would cause specific deficiency syndrome [1,2]. Human have to obtain vitamins from exogenous source such as food where vitamin is one of its natural components and presents in small amount [1]. This is because most vitamins are not synthesized by human [3].

Most plants and animals have the ability to synthesize Vitamin C from glucose [4]. In amphibians and reptiles, Vitamin C synthesis occurs in the kidney and it occurs in the liver for mammals [5]. However, humans, other primates, and guinea pigs cannot synthesize Vitamin C due to the lack of L-gulono-γ-oxidase enzyme which is required in the last step of Vitamin C synthesis [4,5]. Woodall and Ames (as cited in Mohammed *et al*., 2009) commented that mutation in the gene that codes for the particular enzyme is responsible for the loss of aforementioned ability [6]. Due to the inability to synthesize Vitamin C, Vitamin C is acquired through natural source instead such as food [4]. About 90% of Vitamin C in an individual's dietary requirement comes from the consumption of fruits and vegetables [7]. Table 1 shows Vitamin C level in different fruits that had been reported in some selected studies.

Water-soluble vitamin such as Vitamin C is generally more unstable than fat-soluble vitamins such as Vitamins K, A, D, and E [13]. There are several factors that influence the degradation of Vitamin C including heat, light, oxygen, pH, and trace metal ions [14,15]. These factors escalate the reduction of Vitamin C or ascorbic acid to dehydroascorbic acid (DHAA) which can be irreversibly degraded to 2,3-diketogulonic acid by hydrolytic opening of the lactone ring. 2,3-diketogulonic acid is not biologically active [1]. This degradation pathway can occur with or without the presence of oxygen. When Vitamin C in food is concerned, the aerobic degradation mostly occurs during processing of food, while anaerobic degradation is primarily transpired during storage [14].

Moreover, Ghosh *et al.* (2013) cited that leaching causes the most loss of Vitamin C [13]. Leaching means to dissolve out soluble constituents by percolation. This is due to the water-soluble nature of Vitamin C which makes it easy to leak into the surrounding medium. For example, potatoes could lose 40% of its Vitamin C during boiling [1]. Other than that, the ways the foods are being handled also contribute to the decrease of Vitamin C stability including bruising, peeling, cutting into pieces, and exposure to air [7,16]. It was reported that peeling apples could result in the loss of 8–25% of their Vitamin C [16]. Due to its instability, Vitamin C is recognized as an indicator to evaluate the influence of food processing and preservation of vitamin content and is used to estimate certain product's shelf life such as juice concentrate [14].

Researchers studied Vitamin C stability based on the percentage loss of Vitamin C [8,17]. Accordingly, low percentage loss indicates high stability of Vitamin C, while high percentage loss signifies low stability. The effect of heat or temperature on Vitamin C stability in solution was studied, and it showed that when Vitamin C is heated at 30°C, 50°C, 70°C, and 90°C, the retention of Vitamin C was found to be 87.6%, 84.7%, 73.8%, and 43.6% respectively [17]. The study also showed that there was a significant correlation between the loss of Vitamin C and heat. According to Paul and Ghosh (2013), Vitamin C is easily oxidized by heat due to its highly unsaturated structure [18]. In addition, Vitamin C loss in fruits was also studied and Table 2 shows the summary of the reported results.

Other than temperature, storage can also degrade Vitamin C content in foods [14]. For example, stored potatoes lose 50% of their Vitamin C within 5 months and apples stored for winter can also lose 50% of their Vitamin C [1]. Pacier and Martirosyan (2015) recommended freezing for foods that are not to be consumed immediately after purchasing [19]. Table 3 shows the effect of storage period on Vitamin C stability in different fruits reported in several studies.

Table 1: Vitamin C level (mg/100 ml) in some selected fruits

Fruit	Source of fruits (Country)	Vitamin C level (mg/100 ml)	Sources
Orange	Pakistan	12.78	Tareen *et al*., 2015 [8,23]
	Damaturu, Nigeria	39.75±1.00	
	Ethiopia	141.34±22.07	
Pomelo	Nepal	56.93±2.13*	[10]
		66.7±1.72**	
		60.25±0.25**	
Papaya	Pakistan	9.31	Tareen *et al*., 2015 [23]
	Ethiopia	1673±136	
Tomato	Damaturu, Nigeria	19.13±1.63	[8,11]
	Nsukka, Nigeria	37.85±0.06	
Watermelon	Damaturu, Nigeria	27.50±1.25	[8,12]
	Nasarawa, Nigeria	4.08±0.12	

*Using iodometric method, **using dye-titration method, ***using spectrophotometric method

Table 2: Effect of temperature on Vitamin C loss

Food Item	Heating condition		Vitamin C loss (%)	Sources
	Temperature (°C)	Duration (min)		
Orange	20	10	0.9	Diengdoh *et al*., 2014
	50		42.4	
Pineapple	20	10	0.4	
	50		74.3	
Strawberry	20	10	0.2	
	50		48.8	
Grapefruit	35	60	8.2	[16]
	55		13.7	
Pomegranate	70	60	16.1	[18]
	80		18.4	

Table 3: Effect of storage period on Vitamin C loss

Fruits	Storage condition		Vitamin C loss (%)	Sources
	Temperature°C	Duration/period		
Lemon	4–5	1 day	14.3	[7]
		3 days	22.1	
Lime	4–5	1 day	4.7	
		3 days	17.6	
Pineapple	−17	2 weeks	15	[20]
		4 weeks	25.9	
Guava	−17	2 weeks	28.8	
		4 weeks	34.7	
Boabab	−17	2 weeks	8.7	
		4 weeks	12	
Strawberry (heritage)	−20	90 days	20	[21]
		365 days	34	
Strawberry (autumn bliss)	−20	90 days	20	
		365 days	56	

Five different fruits were selected in this study to test for their level of Vitamin C by using the indophenol method. This study also investigated the Vitamin C stability when extracted fruit juices are stored for 7 days at 4°C. Therefore, the aims of the research were as follows:

- To determine the level of Vitamin C in selected fruits (day 0).
- To investigate the effect of storage on Vitamin C and relate it to the stability of Vitamin C in the selected fruits.
- To give recommendation on the optimum time of fresh fruit juice consumption to obtain maximal Vitamin C.

METHODS

Fruits that were tested in this study were orange (*Citrus sinensis*), pomelo *(Citrus maxima)*, papaya (*Carica papaya*), tomato (*Solanum lycopersicum*), and watermelon (*Citrullus lanatus*).

All samples with exception of orange were bought from the wet market at Gadong (*Pasar Gadong*) and they were claimed to be grown locally. Oranges were bought from local supermarket and they were imported from the United States of America. All the samples were washed minimally with tap water to remove dirt before the extraction process begins.

Fruits were bought and processed on the same day. The fruit was peeled and its flesh was cut into smaller pieces to ease the juice extraction process. Slices of the fruit flesh were wrapped with cotton cloth and hand squeezed to extract its juice into a 250 ml beaker [8]. The cotton cloth acted as filter so that the extracted juice would be devoid of pulps. It is to be noted that the extraction was carried out with lights switched off to limit light exposure.

About 10 ml of extracted juice was transferred from the beaker into 50 ml Falcon tubes fully covered with aluminum foil to protect from light and was prelabeled (day 0, 1, 2, 3, and 7). Juice in the tube labeled day 0 was tested for its Vitamin C level on the same day of juice extraction. All the other tubes were covered with aluminum foil to avoid light exposure and then stored in a fridge set at 4°C until it was used for analysis at the intended time.

For the analysis of Vitamin C, 10 ml of 10% trichloroacetic acid (TCA) (Sigma-Aldrich) was then added into the tube containing the juice. The tube must remain covered with aluminum foil. The solution was mixed by placing the tube on the roller mixer for at least 15 min. After it was homogenized, it was subjected to centrifugation at 4700 rpg for 20 min at 4°C to obtain a clear supernatant. The supernatant was finally transferred into 15 ml Falcon tube. This supernatant was used to determine the level of Vitamin C using the indophenol method. In addition to the juice samples being tested, a control solution which was made up of 8 mg ascorbic acid diluted in 10 ml of 10% TCA was also tested for Vitamin C content. Finally, the protocol of indophenol method used in this study was adopted from Food Analysis Laboratory Manual by Nielsen [22] with slight modification [22]. The protocol of recovery assay was adopted from Thermo Fisher Scientific (2007).

RESULTS

The Vitamin C level in each fruit tested is tabulated in Table 4, expressed in mean of the readings ± standard deviation (SD). Orange had the highest level of Vitamin C followed closely by papaya, pomelo, and tomato. Meanwhile, watermelon had the lowest Vitamin C content. The SD of the result of each fruit was also calculated and found to be <1.5. Table 5 shows the Vitamin C content in each fruit on a respective day. All five fruits and the control showed a decrease in Vitamin C by day 7; however, the degree of Vitamin C loss between the fruits was varied. Watermelon already experienced 13.4% loss of Vitamin C on day 1 and the loss continued to increase up to 82.1% on day 7. On the other hand, orange was found to be the one with the highest Vitamin C retention of the five fruits. Orange only experienced 19.2% loss of its original Vitamin C content within 7 days' period. The rank of fruits based on their Vitamin C is a loss which is in this order: Watermelon (82.1%), tomato (70%), pomelo (60%), papaya (28.3%), and orange (19.2%). Finally, control solution lost 37.2% of its Vitamin C on day 7.

The results showed that, for the first 2 days, Vitamin C in control solution remained stable and then decreased afterward. In addition, there was a sharp decrease on day 7. It is observed that pomelo and tomato followed this "control" trend. However, the decline of Vitamin C in the other three fruits orange, papaya, and watermelon did not share this pattern, but they had similar degradation trend to each other. All three fruits experienced a reduction in Vitamin C level on day 1 and shared a noticeable reduction on day 2 as well. Their levels continued to decrease until the end of the 7 days' storage period.

Furthermore, the data on day 0 and 7 were compared and a paired t-test was performed by Microsoft Excel. The paired t-test showed $p<0.05$ for all the fruits which means that the loss of Vitamin C is significant. This signifies that there is a significant correlation between loss of Vitamin C in all the five fruits and storage period. For the recovery assay analysis, at lower spike level (36 mg/100 ml), the recovery percentages were relatively close to 100%, while higher spike level (136 mg/100 ml) produced higher recovery percentage (Table 6). All values are expressed in mean of three readings ± SD.

Table 4: Vitamin C level (mg/100 ml) for each fruit

Fruit	Vitamin C level Mean±SD (mg/100 ml)
Orange	58.2±0.00
Pomelo	54.0±0.73
Papaya	56.9±0.73
Tomato	22.4±1.46
Watermelon	6.7±1.27

All data are expressed as mean±SD of a triplicate set of values (n=3).
SD: Standard deviation

DISCUSSION

The result of this study was compared with other previous studies and the levels of Vitamin C reported were varied. A study conducted by Tareen *et al.* [23] found that orange had 12.78 mg/100 ml of Vitamin C [23]. El-Ishaq and Obirinakem (2015) reported that the Vitamin C content in orange was 39.75±1.00 mg/100 ml [8]. Another study by Bekele and Geleta [9] found much higher content of Vitamin C in orange which was 141.34±22.07 mg/100 mL [9]. However, in this present study, orange had 58.2±0.00 mg/100 ml of Vitamin C. The oranges used in the previous studies were of different origins. Oranges studied by Tareen *et al.* [23], El-Ishaq and Obirinakem (2015), and Bekele and Geleta (2015) were from Pakistan, Nigeria, and Ethiopia, respectively [8,9]. This shows that the location of where the fruits were obtained greatly influences the level of Vitamin C.

In this study, pomelo had 54.0±0.73 mg/100 ml of Vitamin C. This value was in agreement with the value reported by Shrestha *et al*. [10] using iodometric titration which was 56.93±2.13 mg/100 ml [10]. However, the authors also carried out the determination of Vitamin C using dye titration and spectrophotometric method. They found that Vitamin C level in Pomelo in Nepal to be 66.7±1.72 mg/100 ml using the dye titration method and 60.25±0.25 mg/100 ml when using the spectophotometric method. Their study depicts that the method of analysis could give variance in the result.

Bekele and Greta [9] reported that Vitamin C level in papaya was 1673±136 mg/100 mL; however, this present study reported much lower result of 56.9±0.73 mg/100 ml [9]. Tareen *et al.* (2015) reported even lower result in which the level of Vitamin C in papaya was 9.31 mg/100 ml [23]. The difference could again be attributed to the difference in location. Papaya studied by Tareen *et al.* (2015) and Bekele and Greta [9] was from Pakistan and Ethiopia, respectively.

The Vitamin C content of tomato in this study was found to be 22.4±1.46 mg/100 ml. This value was close to the value reported by El-Ishaq and Obirinakem (2015) which was 19.13±1.63 mg/100ml [8]. However, Madukwe *et al*. [11] reported a higher level which was 37.85±0.06 mg/100 ml [11]. Both studies used tomato originated in Nigeria but from different regions. Tomato studied by El-Ishaq and Obirinakem (2015) and Madukwe *et al*. (2013) was from Damaturu and Nsukka, Nigeria, respectively [8,11].

Table 5: Vitamin C level (mg/100 ml) over 7 days

Fruit/Sample	Vitamin C level Mean±SD (mg/100 ml)				
	Day 0	Day 1	Day 2	Day 3	Day 7
Orange	58.2±0.00	56.8±1.23	53.4±0.71	51.3±0.00	47.0±0.72
Pomelo	54.0±0.73	53.9±0.71	52.5±0.71	51.3±1.25	21.6±2.60
Papaya	56.9±0.73	53.5±0.71	46.8±0.71	45.5±1.44	40.8±1.91
Tomato	22.4±1.46	20.6±0.71	20.5±0.00	17.1±0.72	6.7±0.00
Watermelon	6.7±1.27	5.8±1.43	2.5±0.00	1.7±1.91	1.2±0.72
Control	74.2±0.97	74.0±0.36	72.9±0.36	68.4±0.96	46.6±0.72

All data are expressed as mean±SD of a triplicate set of values (n=3). SD: Standard deviation

Table 6: Percentage recovery in each fruit in different spike levels

Fruit	Spike level (36 mg/100 ml)			Spike level (136 mg/100 ml)		
	Expected value (mg/100 ml)	Observed value (mg/100 ml)	Recovery (%)	Expected value (mg/100 ml)	Observed value (mg/100 ml)	Recovery (%)
Orange	38.4±0.8	33.7±0.7	87.8	132±0.8	134±2.5	101.5
Pomelo		33.9±2.2	88.3		130±0.7	98.5
Papaya		34.4±0.4	89.6		137±0.4	103.8
Tomato		38.2±0.7	99.5		147±5.8	111.4
Watermelon		37.7±0.0	98.2		141±1.4	106.8

All data are expressed as mean±SD of a triplicate set of values (n=3). SD: Standard deviation

Similar observation was made in comparing the values of Vitamin C in watermelon. The level of Vitamin C in watermelon in this present study which was 6.7±1.27 mg/100 ml was comparable to the value reported by Nweze *et al*. (2015) which was 4.08±0.12 mg/100 ml [12]. However, the level of Vitamin C in watermelon reported by El-Ishaq and Obirinakem (2015) was higher (27.50±1.25 mg/100 ml). Both of the previous studies used watermelon from different regions as well [8]. Watermelon studied by El-Ishaq and Obirinakem (2015) and Nweze *et al*. (2015) was from Damaturu and Nasarawa, Nigeria, respectively [8,12]. These comparisons emphasized that regional location of fruit is a key factor in explaining the difference in the Vitamin C level. In addition, a study by Wall [24] found that papaya of the same cultivar acquired from two different regions in Hawaii had a difference of 11.4 mg/100 g [24].

Some of the studies mentioned above employed different methods to determine Vitamin C level than the method used in this present study. It could be speculated that the method may contribute to the result variation observed. However, Esch *et al*. (2010) compared the results of their Vitamin C level by cyclic voltammetry and 2,6-dichlorophenolindophenol titration and they found no significant difference [25].

Even when the same methodology is applied, there is still a considerable difference in the level of Vitamin C in the same species of fruit. For example, El-Ishaq and Obirinakem (2015) found that orange had 39.75±1.00 mg/100 ml, while this present study found the Vitamin C level in orange to be 58.2±0.00 mg/100 ml [8]. Both studies used indophenol method for Vitamin C determination. It was reported in multiple studies that this observed difference is due to several factors which include ripening stage and regional varieties of fruits, types and storage period, and climatic conditions such as temperature and light [9,10]. In addition to these factors, the amount of nitrogen fertilizers used can also affect Vitamin C content. Increasing the amount from 80 to 120 kg/ha decreased the Vitamin C content by 7% in cauliflower [25]. However, whether the fruits are grown organically or conventionally proved to have no effect on the level of Vitamin C.

It needs to be taken into account that the level of Vitamin C could even vary between the different fruit samples of the same species [9,23] and this was supported by the pattern observed in this study. For instance, the level of different papaya sample varied around 11.9 mg/100ml. Another factor that could account to the difference in Vitamin C level is the position of the fruit on the tree. According to Davey *et al*. (as cited in El-Ishaq and Obirinakem, 2015), fruits located on the outside of the tree and at the south side have higher Vitamin C content, while fruits that are located inside and shaded have lower content [26].

CONCLUSIONS

Although the trends were similar, the degree of Vitamin C loss was different. Overall, orange had the least percentage loss of 19.2%, while watermelon had the highest percentage loss of 82.1%. Based on these findings, Vitamin C in orange is the most stable out of the five fruits.

The result of this study was compared with the study conducted by El-Ishaq and Obirinakem (2015). They reported that tomato and watermelon had a percentage loss of 18% and 21.3%, respectively. However, this present study found that tomato and watermelon had a percentage loss of 70% and 82.1%, respectively. Unfortunately, they did not specify the temperature of the storage where the juice was kept for 7 days.

Other than that, Spinola *et al.* (2012) reported in their study that Vitamin C remained stable for at least 24 h when stored at 4°C and without exposure to daylight [27]. They added that, after 24 h, there was a distinguished decline in Vitamin C content in their samples. Their observations were in agreement to what was observed in this present study where Vitamin C in orange and pomelo shown to have stability for 24 h. They further recommended that, if sample is to be stored for 1 week or less, −20°C would be the ideal temperature as it had shown a lesser degree of Vitamin C loss.

Moreover, this present study determined that the Vitamin C loss in watermelon was 82.1%. However, a study by Ibrahim [28] reported Vitamin C loss of only 5.6% in watermelon after 1 week storage at 4°C–5°C [28]. The author stated that the watermelon extract was added with sugar solution before it was stored. Hence, addition of sugar may contribute to the retention of Vitamin C. This is in agreement to the result reported by Sapeia and Hwaa [29] where they concluded that addition of sugar slows down the degradation of Vitamin C [29].

El-Ishaq and Obirinakem (2015) provided an explanation that the loss of Vitamin C during storage was due to the chemical reactions [8]. Most of these reactions are catalyzed by enzymes and the others occurred simply due to senescence. These reactions occurred between the constituents of the fruits that would eventually cause color, flavor, and odor changes. Ultimately, it resulted in the change of pH.

The ideal result for recovery assay is 100% recovery which indicates that the assay generates a result with high accuracy. Based on the findings of this study, in lower spike level, all percentage recoveries were below 100% and this suggests that, when measuring relatively low level of Vitamin C in a sample, this assay had the tendency to underestimate the level of Vitamin C. On the other hand, when measuring relatively higher level of Vitamin C, this assay overestimates the Vitamin C level. This is based on the findings of recovery assay where all of percentage recovery except for pomelo surpassed 100%. However, indophenol method is still a recognized AOCA method and is still used in many recent studies [7,8,16].

Another limitation concerns with the indophenol method employed in this study specifically the determination of endpoint. The determination was heavily relied on the operator's judgment, and therefore, it is very much subjective. This could have an impact on the results of Vitamin C level. In addition to that, this method does not include and calculated DHAA level. The absence of DHAA level value causes total Vitamin C level in the fruit to be underestimated [27].

Finally, there were a lot of uncertainty factors in the pre-analytical stage of this study specifically the origin of fruit sample. Factors such as maturity stage of the fruit tested, storage conditions of the fruits employed by the vendors, and the growing conditions of the fruits such as amount of nitrogen fertilizers used were pretty much unknown. One possible solution to this is to plant own fruits where the aforementioned factors that could affect the level of Vitamin C are known and could be controlled.

First, other extraction methods could be tested including agitation extraction, ultrasound extraction, high hydrostatic pressure extraction, or other conventional methods such as using fruit presser or blender with the presence of extracting solution [30]. These methods could then be compared and identified which extraction method could minimize the degree of Vitamin C loss.

Second, other methods of determination of Vitamin C could be performed. This includes high-performance liquid chromatography, spectrophotometric assay, and cyclic voltammetry [25,27,31]. These methods could then be compared based on the results produced, and advantageous or disadvantageous of a certain method could also be identified. Moreover, the duration of the storage period could be extended 1 more week or 2 to investigate the total degradation of Vitamin C. Since this study focused on the effect of storage duration on Vitamin C stability, perhaps, other factors that affect Vitamin C stability such as metal ions, temperature, light, and pH could be investigated as well. Consequently, factor that causes a high level of Vitamin C loss could be determined. In addition, samples could be subjected to different combinations of factors, for example, light and temperature and thus assess which combination and to what degree it is “lethal” to Vitamin C.

This study concluded that, of the five selected fruits (orange, pomelo, papaya, tomato, and watermelon), orange had the highest level of Vitamin C (58.2±0.00 mg/100 ml), while watermelon contained the least (6.7±1.27 mg/100 ml). Furthermore, following the findings of the effect of storage on Vitamin C stability, it can be concluded that Vitamin C in orange is the most stable of all five. Orange experienced Vitamin C loss of 19.2%, while Vitamin C in the watermelon was easily degraded in which 82.1% of Vitamin C was lost after 7 days storage. Furthermore, a comparison of data on day 0 and day 7 showed that the difference in the level of Vitamin C is significant. This means that storage duration has significantly affect Vitamin C stability. The result of the stability study also showed that Vitamin C in orange and pomelo was stable for at least 24 h when appropriately stored at 4°C. Hence, this study recommends fresh fruit juice; particularly, watermelon is best consumed within 24 h to get the most Vitamin C. This is important because Vitamin C is one of the essential micronutrients that our body needs daily. It serves a lot of biological functions and offers many health benefits to individuals. Alas, humans do not have the ability to synthesize Vitamin C and thus have to consume fruits or vegetables to get Vitamin C.

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CONFLICTS OF INTEREST

Authors wish to declare no conflicts of interest.

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