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The Effect of Storage Method on the Vitamin C Content in Some Tropical Fruit Juices

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Abstract: Loss in vitamin C contents of some fruit juices namely, orange, lemon, lime, pineapple, paw-paw and carrot stored under different conditions was investigated. The juice from the fruit samples were extracted, stored at room temperature (29±1°C) in plastic bottles and in the refrigerator (4±1°C) for 4 weeks. The juices were all analysed for their vitamin C content by oxidation and reduction method. Results revealed that the rate at which vitamin C is lost during storage depends on the type of fruit and the storage method employed. The citrus fruits were found to follow a similar pattern of loss, while other fruits differ from this and among themselves. Loss of vitamin C correlated with pH only for pineapple, pawpaw and carrot, however, this cannot be said to be the controlling factor. *Bacillus subtilis* and *Candida* sp. were isolated from all the juices under both storage conditions, except for orange juice.

Key words: Vitamin C, loss microbes, refrigeration, room temperature

INTRODUCTION

Vitamin C (also referred to as L-ascorbic acid) is the lactone 2,3-dienol-L-gluconic acid and it belongs to the water-soluble class of vitamins. Ascorbic acid is an odourless, white solid having the chemical formula $C_6H_8O_6$. Vitamin C is mainly found in fruits and vegetables. In the nutritional content, vitamin C is the L-enantiomic form of ascorbic acid which also encompasses the oxidation product of dehydroascorbic acid with different oxidizing agent. It participates in numerous biochemical reactions, suggesting that vitamin C is important for every body process from bone formation to scar tissue repair (Rickman *et al*., 2007). The only established role of the vitamin C appears to be in curing or preventing scurvy and it is the major water-soluble antioxidant within the body.

Factors that affect the vitamin C contents of citrus fruits include, production factors and climate conditions, maturity state and position on the tree, type of fruits (species and variety), handling and storage, type of container (Naggy, 1980). Immature fruit has the highest levels and decreases during the ripening process. Early maturing varieties have higher levels than late maturing types. High nitrogen fertilizer rates can lower vitamin C levels in citrus fruits. Proper potassium levels are also needed for good vitamin C level (Padayatty *et al*., 2003).

Pasteur identified the growth micro organisms such as bacteria and fungi as the scientific cause of spoilage and decay in the 1860s, other causes include chemical changes from ripening and senescence (aging) processes occurring in the fruit. Bacteria and fungi are everywhere in our environment and most foods provide an excellent substrate (http://www.answers.com/topic/substrate) for their growth (Manso *et al*., 2001). Vitamin C bears an obvious structural similarity with hexose sugars; hence, it is conceivable that the molecule might serve as a carbon source for respiration or bacterial growth that it might be fermented (Eddy and Ingram, 1953). Storage conditions of low temperature and humidity have been found to retard microbial growth; chemical and biological processes are also slowed down

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(Manso *et al*., 2001 actahort.org/books/566/index.htm). However, once these protective barriers are breached, microbial growth is often unchecked and rapidly destroys the commodity. The flavour, texture and nutrition of many fruits and vegetables are reduced before visual appearance of spoilage (María Gil *et al*., 2006).

Oxygen is the most destructive ingredient in juice causing degradation of vitamin C. However, one of the major sugar found in orange juice, fructose, can also cause vitamin C breakdown. The higher the fructose content, the greater the loss of vitamin C. Conversely, higher acid level of citric acid and malic acid stabilize vitamin C (Padayatty *et al*., 2003). Canned juices are often regarded as less nutritious than fresh or frozen products therefore the preference for fresh/preserved fruits in this country. This necessitated this study on the effects of storage on the quality of some common fruits using vitamin C as the reference.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh fruits of *Citrus sinensis* (orange), *Citrus limon* (lemon), *Citrus aurantifolia* (lime), *Ananus comosus* (pineapple), *Asimina triloba* (pawpaw) and carrot were purchased from retail outlets in Zaria, a Northern Nigerian city. The study was carried out in Ahmadu Bello Univeristy, Zaria-Nigeria between March and June 2007. These fruits were washed thoroughly with water and the juices were extracted by mechanical pressure. Each type of juice samples was filtered to remove pulp and seeds and stored in already labelled plastic containers.

Reagents

All chemicals used were obtained from BDH London, unless otherwise stated were of analytical grade purity and double distilled water was used.

One percent starch indicator solution was prepared by adding 0.50 g of soluble starch in 50 mL of near-boiling water.

Iodine solution was prepared by dissolving 5.0 g of potassium iodide (KI) and 0.268 g of potassium iodate (KIO_3) in 200 mL of water followed by addition of 3 M sulphuric acid. The solution was made up to 500 mL in a graduated cylinder and then transferred to a beaker.

Vitamin C standard solution was prepared by dissolving 0.250 g of vitamin C in 100 mL of water and then diluted to 250 mL with water in a volumetric flask.

Vitamin C Determination by Iodine Titration

Oxidation-reduction method described by Helmenstine (2008)(http:// www.chemistry. about.com) was used.

Standardizing Solutions and Titration of Juice Samples

Vitamin C solution (25 mL) was transferred into 100 mL conical flask and 10 drops of starch solution was added. This was titrated with the iodine solution until the first blue colour which persisted for about 20 sec was observed. Juice samples (25 mL) were titrated exactly the same way as the standard. The initial and final volume of iodine solution required to produce the colour change at the endpoint was recorded. Titration was performed in triplicate in all cases.

Microbial Test

The samples were cultured on blood agar medium, incubated at 37°C for 24 h, the colonies of the organisms were gram stained, biochemical tests were carried out to identify the bacteria, according to the method described by Singleton (1999). The yeast identification was performed with fluoroplate candida agar according to the method of Manafi and Willinger (1991).

RESULTS AND DISCUSSION

The retention of vitamin C is often used as an estimate for the overall nutrient retention of food products because it is by far the least stable nutrient; it is highly sensitive to oxidation and leaching into water-soluble media during storage (Davey *et al*., 2000; Franke *et al*., 2004). It begins to degrade immediately after harvest and degrades steadily during prolonged storage (Murcia *et al*., 2000) and also continues to degrade during prolonged storage of frozen products (Rickman *et al*., 2007). Results for the freshly squeezed fruits shows that the oranges had the highest vitamin C content, followed by lemons, limes, pineapple, pawpaw and carrot. The values obtained for citrus fruits are quite lower than values obtained elsewhere (http://www.naturalhub.com/natural_food_guide_fruit_vitamin_c.htm). This is consistent with reports that, climate, especially temperature affect vitamin C level. Areas with cool nights produce citrus fruits with higher vitamin C levels. Hot tropical areas produce fruit with lower levels of vitamin C (Padayatty *et al*., 2003). Also, environmental conditions that increase the acidity of citrus fruits also increase vitamin C levels.

The results have shown that the environment in which juice is stored can affect its vitamin C content significantly (Fig. 1). The pattern of loss in vitamin C showed an initial increase in the first two weeks followed by decrease in orange samples RT. The RC samples decreased initially, followed by an increase and then a decrease. The concentration of vitamin C decreased faster in RC than in RT samples, however, the same pattern was observed throughout the four weeks of storage. The reason for the initial increases is not understood, but Rickman *et al*. (2007) attributed this to a change in moisture content during the storage of frozen peas.

The trend in the concentration of vitamin C for the lemon samples, over the period of investigation is similar to that observed for oranges. There was an initial decrease, then an increase at two weeks and then a decrease. The difference in the concentration of vitamin C between RT and RC at any particular time is not much. The result also showed that more vitamin C is lost in lemon over this period than in oranges. For the lime sample the pattern of decrease differ slightly for the RC samples. The initial increase in the vitamin C content was not observed for RC samples. However, like the orange and lemon the RC samples lost more vitamin C than the RT samples.

Light exposure was found to promote browning in pineapple juice. Ten percent losses in vitamin C have been reported after 6 days at 5°C in pineapple pieces by María Gil *et al*. (2006). Pineapple samples showed a different pattern of decrease in vitamin C content compared to the citrus fruits. Here, the RC samples retained more vitamin C than the RT samples after four weeks of storage. The initial increase in the vitamin C content observed in the citrus fruits was not observed with the pineapple sample. This suggests that variation in moisture content cannot be the sole controlling factor leading to the initial increase observed in the citrus fruits. Again no reason can be proffered from this investigation why the retention of vitamin C is more in the RC samples than in the RT samples. Since, vitamin C is unstable in neutral and alkaline environments therefore the longer the exposure, the greater the loss of vitamin C. The increase in pH (Table 1) was related to deterioration of fruit characteristics (María Gil *et al*., 2006).

The RT pawpaw sample showed a rapid initial decrease in vitamin C content within the first two weeks. At this period the RC sample showed a steady decrease with vitamin C content higher than RT. By the third week Vitamin C content in RT increased above RC after which, both RT and RC decreased very rapidly, with RC tending towards zero vitamin C content. Finally, in the carrot sample, RT and RC decreased rapidly at first with RC retaining more vitamin C up to the second week. After the second week, difference in vitamin C content between RT and RC became very small, both decreasing till the fourth week.

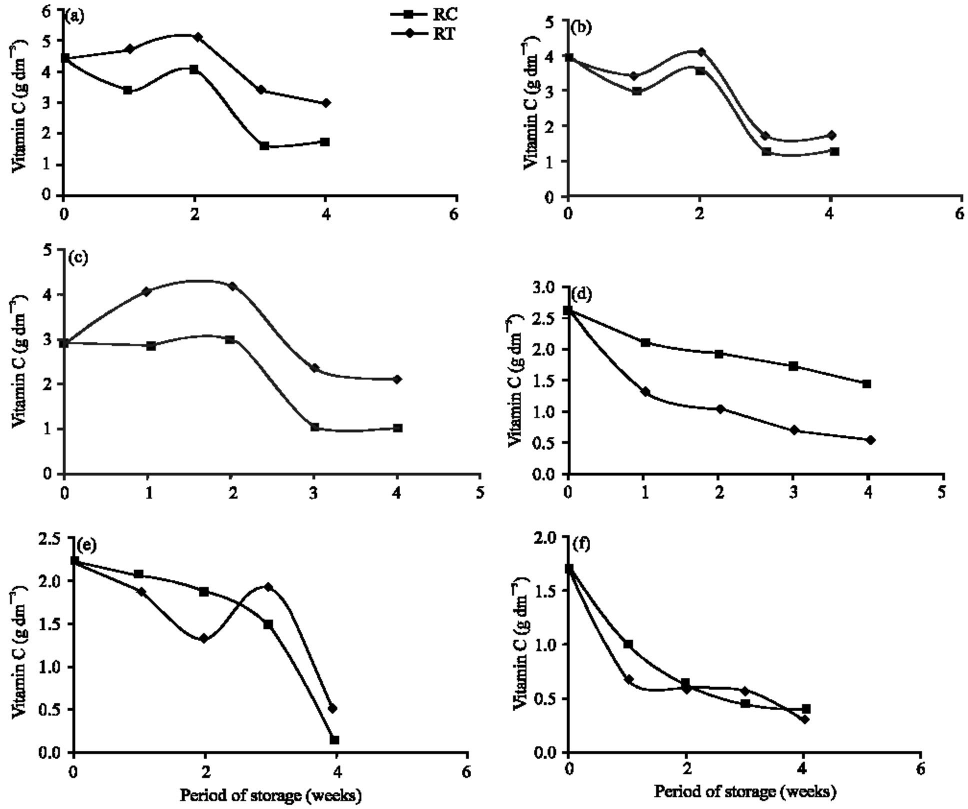

Fig. 1: Variation of vitamin C content of fruits with time and mode of storage (a) Orange, (b) Lemon, (c) Lime, (d) Pineapple, (e) Paw paw and (f) Carrot

Table 1: pH values of the fruit juice with storage time

Storage environment/week	Orange	Lemon	Lime	Pineapple	Paw paw	Carrot
Fresh	4.65	3.15	3.30	5.53	5.87	6.29
RT1	4.55	3.01	2.91	5.82	5.88	6.48
RT2	4.49	3.28	3.00	5.92	5.96	6.78
RT3	4.68	3.45	3.04	6.18	6.19	6.85
RT4	4.78	3.26	3.09	6.93	6.70	6.97
RC1	4.35	3.19	2.85	5.44	5.53	5.82
RC2	4.20	3.08	2.92	5.96	5.71	6.07
RC3	4.50	3.28	3.06	6.29	6.02	6.81
RC4	4.51	3.31	3.05	6.41	6.27	6.98

RT: Room Temperature; RC: Refrigerated at 4°C

Many chemical reactions contribute to the loss of storage life of vitamin C and hence chemical deterioration of fruits. The majority of these reactions are enzymatically driven while others are chemical reactions that occur because of the senescence (aging) processes. This involves colour, flavour, and odour changes that result from a chemical reaction between the constituents of the fruit. Fruit can be a vector and provide a growth medium for many pathogenic microbes which can produce potent toxins. In this study *Bacillus subtilis* and *Candida* sp. were isolated from both RT and RC of all the fruits used in this investigation, except in orange where only *Candida* sp. was isolated. *Bacillus subtilis* is not considered a human pathogen; it produces the proteolytic enzyme subtilisin (a protein-digesting enzyme) and has been implicated in food poisoning and spoilage (Ryan and Sherris, 1994). *Candida albicans* sp. (yeast) has been reported as the causative agent of spoilage of sugary foods, such as condensed milk, fruit juices and concentrates (Stratford *et al*., 2002). The biochemical reactions occurring over the storage period together with microbial action in all fruit juices resulted in pH changes observed (Table 1). Two-tailed Spearman's correlation showed that there is a significant correlation between pH and vitamin C at 95% confidence level for RT samples of pineapple ($r^2 = 0.74$), pawpaw ($r^2 = 0.84$) and carrot ($r^2 = 0.75$). For RC samples only pineapple ($r^2 = 0.77$) and pawpaw ($r^2 = 0.70$) showed a significant correlation. This result shows that pH is also not the sole controlling factor in the deterioration of vitamin C in fruit juice with storage life.

CONCLUSION

This study supports the common perception that fresh is often best for optimal vitamin C content, as long as the fresh product undergoes minimal storage at either room or refrigerated temperatures. Loss of vitamin with time differs from one fruit to the other under similar storage environments. While the refrigerated samples cause significant loss of ascorbic acid in the citrus fruits, this is not so in pineapple, pawpaw and carrot samples. Though pH is significant in the stability of vitamin C, it cannot be said to be the sole controlling factor leading to losses observed in all the fruits investigated.

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