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THERMAL INFLUENCE ON THE STABILITY OF L-ASCORBIC ACID FROM SELECTED EXOTIC TROPICAL FRUITS

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ABSTRACT

The thermal stability of ascorbic acid in some exotic tropical fruits like pineapple, orange, lemon, lime, grape and mango at various temperatures as monitored using the 2, 4-dinitrophenyl hydrazine (DNPH) method showed a gradual decline of ascorbic acid content of these fruits with increase in temperature from 40°C to 80°C suggesting that the water soluble acid is heat sensitive. Pineapple declined from 15.50mg/100ml of extract at 40°C to 0.2mg/100ml at 80°C, orange declined from 53.70mg/100ml to 0.30 mg/100ml, lemon declined from 46.20mg/100ml to 0.90 mg/100ml, lime decline from 29.0mg/100ml to 0.50 mg/100ml whereas grape and mango declined from 34.70mg/100ml and 27.40mg/100ml to 0.90 mg/100ml and 0.50 mg/100ml respectively. The stability profile also showed that the rate of denaturation of this acid in the various fruits differ at each temperature as shown in their stability profiles indicating that the fruits have differing abilities to resist thermal induced degradation. The decline in the stability of ascorbic acid of these exotic tropical fruits may be due to the sensitivity of the lactone ring of ascorbic acid to high temperature.

KEY WORDS: *Ascorbic acid, pineapple, orange, lime, lemon, grape, mango.*

INTRODUCTION

Ascorbic acid, a naturally occurring six carbon member compound with a heterocyclic ring is a water soluble compound having enviable antioxidant properties. The pure form has whitish appearance whereas the impure form is yellowish. Both however are soluble in water giving a slightly acidic solution. The acid is derived from glucose and so many animals like the vertebrates are able to produce it whereas primates like man, guinea pigs, bats and birds cannot. The must obtain it as micronutrient from external source Lachapelle, (2011). L-Ascorbic acid, most commonly referred to as Vitamin C, is the edible isomer of the ascorbic acid which gained its popularity due to its anti-oxidant properties. The molecule consist of a five membered heterocyclic ring with oxygen as the hetero-atom and four hydroxyl groups of which two are attached to the ring and the other two attached to an ethyl branch. This acid derives its properties mainly from the structure and oxidizes rapidly to prevent the destruction of cells in the body by foreign agents like free radicals. It must be stored in cool dark environment free from metals Chatterjee, (1979). Goats, like most animals synthesize their own vitamin C and a 70kg goat can manufacture more than 13,000mg of vitamin C per day in normal health and many times higher when in stress Chatterjee, (1973). The best sources of vitamin C are citrus fruits and other juices in fruits such as oranges, strawberries,

bananas, grapefruit and water melon. A wide variety of other foods contains

sufficient quantities of vitamin C such as cabbage, tomatoes, potatoes, beans, lettuce, broccoli etc. Jacob, (1999).

It has been observed that the muscle provides the majority of meat consumed in diet among tropical inhabitants, and that meats are prepared at high temperatures as in cooking, roasting etc, animal products are not reliable source of vitamin C. This vitamin has been found to be present in human milk but not in raw cow milk Clark, (2007).

Ascorbic acid is produced industrially from glucose using the method according to Tadeus Reichstein "Reichstein Process." In a five step process, glucose is catalytically hydrogenated to sorbitol which is oxidized by microorganism (*Acetobacter suboxydans*) to sorbose. Oxidation occurs only on one hydroxyl group but treatment of the product with acetone in the presence of an acid catalyst converts the other four hydroxyls to acetals. The ring closure into lactone is achieved by acid catalyzed hydrolysis together with the removal of the two acetal groups with a 90% yield at each step Christopher, Ute, Stephanie and Hermann, (2006). Among the animals that have lost the ability to produce ascorbic acid on their own are the Simians and Tarsiers which constitute one of the two major classes of primates (anthropoidae or haplorrhini) which include humans. The other primitive primates (strepsirrhini) do have the ability to synthesize their own ascorbic acid. However a number of passerine birds do not synthesize ascorbic acid Martinez and Carlos, (1997). All tested families of fruit eating bats, mammals (including man) and insects cannot synthesize their own ascorbic acid owing to the lack of the enzyme l-gluconolactone oxidase (GULO) which is a major requirement in the last stage of the synthesis of ascorbic acid because they have a differing pseudo gene (ψGLO) that cannot allow the process Harris and James, (1996). In a survey of fruit eating bats a trace of GULO was detected in only 1 out of 34 bat species tested across the range of 6 families Jenness, Birney and Ayaz (1980). Similar non-functional gene is also present in the genome of primates like humans and guinea pigs Nikishimi, Kawai and Yagi, (1992). Some of these species including humans are able to survive with lower concentrations obtained from their foods by recycling ascorbic acid Milton, (1999). Ascorbic acid part from being an antioxidant also show the properties of a pro-oxidant and the ability to reduce d-block metals like copper II (Cu^{2+}) and iron III (Fe^{3+}) ions into copper I (Cu^{+}) and iron II (Feb^{2+}) respectively while it is being converted from the ascorbate to the dehydroascorbate forms in the body Satoh and Sakagami, (1997). Since human beings cannot produce their own ascorbic acids, their ascorbic acid requirements must be generated from the food they eat. This acid helps to build, repair and maintain body connective tissues, breakdown amino acids and with the antioxidant property, protect the body from harmful toxic chemicals.

Research has also shown that freshly cut fruits do not lose much of their nutrients including ascorbic acid when stored at low temperature for few days (Hitti and Miranda, (2006). Ascorbic acid (Laboratory grade), grape juice and vitamin C tablet (a pharmaceutical product) were either packaged in different materials or exposed to air or stored at different temperature were used to study stability of ascorbic acid using titrimetric analytical method with 2,6-dichlorophenolindophenol. Results of analysis showed that packaging materials, exposure to air and storage temperature condition significantly affected the stability of the vitamin. A significant negative correlation exists between ascorbic acid decline and time of storage and of exposure to air. Ascorbic acid content was more stable in the three sources when stored under refrigeration condition (4-5°C) as obtained in the investigation, Oyetade, Oyeleke, Adegoke and Akintunde (2012). The effect of time and temperature on vitamin C stability in horticultural extracts using UHPLC-PDA vs iodometric titration as analytical methods has been investigated Vitor, Bertha, Jose and Paula, (2012). It was found that the amount of ascorbic acid present reduces with increasing temperature. The effect of thermal treatment on the Ascorbic acid content of pomegranate juice has been monitored by Ranu and Uma, (2012). It was shown that the ascorbic acid's anti-oxidant properties declined with increasing temperature until when close to 80°C where there will be no significant property. Also, the ascorbic acid content of three common juicy tropical fruits, orange, water melon and cashew, were determined using iodometric titration method under three temperature regimes (refrigerated, room temperature, and heated to about 80°C, representing the range of temperatures the fruits may be exposed to during processing and storage. It was observed that fruits exposed to higher temperature contained the least of the ascorbic acid. This is as a result of increase in oxidation of ascorbic acid with increase in temperature, as higher temperature favours redox reaction Dioha, Olugbemi, Onuegbu and Shahru, (2011).

Many researchers report different concentrations of ascorbic acid in various fruits and vegetables. The variations in reports have been attributed to a number of factors that may affect the ascorbic acid levels in fruits Dioha et al (2011). These

include climate, temperature and soil nutrients. The composition of plant tissues during growth and development is also determined by temperature which varies from region to region. For example, grapefruits grown in the coastal region of California have been reported to contain more vitamin C than those grown in the desert areas of California and Arizona (Lee and Kader, 2000).

This research however sought to reaffirm the fact that the ascorbic acid content of tropical fruits may be influenced by environmental factors as well as the local practice of not only consuming these tropical fruits raw but also preserving them under low temperature so as to retain the ascorbic acid in them.

Experimental Procedure

The ascorbic acid content of the samples of homogenized samples of pineapple, orange, lemon, lime, grape and mango were oxidized to dehydroascorbic acid by shaking with activated charcoal in the presence of ethanoic acid. After coupling with 2, 4-dinitrophenyl hydrazine (DNPH) the solution was treated with tetraoxosulphate (VI) acid to produce a complex with red colouration measured spectrophotometrically at 540nm according to Jayaraman, (1980).

Sample Preparation

Fresh samples of pineapple, orange, lemon, lime, grape and mango were obtained from a local market in Igbesa, Ogun State, Nigeria. The samples were washed and classified into nine groups for storage, with each group containing five samples of each fruit. The first group of fruits was kept at 40°C to serve as control. The second group of fruits were heated for 10 minutes at a temperature of 45°C and allowed to cool while the third group was heated for 10 minutes at the temperature of 50°C and cooled the same way. The fourth to ninth groups were treated in the same way for 10 minutes at 5°C difference in temperature. The samples were washed again with distilled water, peeled and sliced into two transversely, and thawed. The juice extract recovered for each group was filtered with a muslin cloth and then with Wattman 4" filter paper. The extracts were stored in labeled sample tubes for analysis.

Sample Analysis

The ascorbic acid content of the prepared samples of pineapple, orange, lemon, lime, grape and mango was determined using the 2, 4-dinitrophenyl hydrazine (DNPH) method Jayaraman, (1980).

10ml of each of the fruit sample was homogenized in sufficient 5% metaphosphoric acid / 10% ethanoic acid mixture obtained by dissolving 15g of metaphosphoric acid in a mixture of 40ml of glacial ethanoic acid and 459ml of distilled water. The solution was filtered and stored.

15g of acid washed activated charcoal was mixed with15ml of the stored filtrate and shaken vigorously for proper homogenization. The mixture was again filtered to remove the charcoal and 4.0ml of the filtrate measure out in a test tube. 1 drop of 10% thiourea solution was added to the filtrate in the test tube and subsequently 1ml of 2, 4-dinitrophenyl hydrazine (DNPH) reagent obtained by dissolving 2g of the 2, 4-dinitrophenyl hydrazine (DNPH) in 100ml of 9N H_2SO_4 and filtered). The test tube was placed in a water bath at 37°C for exactly 3hrs. The blank was made without the reagent and equally subjected to the same conditions. The test tube was cooled in ice bath and 5ml of 85% H_2SO_4 (made by dissolving 900ml of conc. H_2SO_4 to 100ml of distilled water) added with stirring. The reagent was then added to the blank. The coloured complex was red at 540nm after 30mins. Also a calibration curve was carried out with ascorbic acid standard in the range of 0.25-15mg /ml.

RESULTS

The ascorbic acid content of the selected fruit juices was analyzed and the results obtained were as below:

Temp ⇒ Fruits(mg/100ml) ⇩	40°C	45°C	50°C	55°C	60°C	65°C	70°C	75°C	80°C
Pineapple	15.50	14.20	12.60	10.90	9.20	7.20	5.10	2.70	0.20
Orange	53.70	48.60	42.10	34.30	26.30	17.90	9.40	1.70	0.30
Lemon	46.20	42.30	38.10	33.50	27.80	21.20	14.10	6.50	0.90
Lime	29.00	26.80	24.20	21.40	18.40	14.10	10.40	5.60	0.50
Grape	34.70	31.30	27.70	24.10	20.10	15.90	11.50	6.60	0.90
Mango	27.40	24.90	22.10	19.00	15.70	12.20	8.50	4.60	0.50

Table 1. Conc. of Ascorbic acid (mg/100ml) of fruits with changes in temperature.

Temp⇨ Fruits(mg/100ml)⇩	40°C - 45°C	45°C - 50°C	50°C - 55°C	55°C - 60°C	60°C - 65°C	65°C - 70°C	70°C - 75°C	75°C - 80°C
Pineapple	1.30	1.60	1.70	1.70	2.00	2.10	2.40	2.50
Orange	5.10	6.60	6.80	7.00	7.40	7.50	7.70	1.40
Lemon	3.90	4.20	4.60	5.70	6.60	7.10	7.60	5.40
Lime	2.20	2.60	2.80	3.00	3.20	4.30	4.80	5.10
Grape	2.40	3.60	3.80	4.00	4.20	4.40	4.90	5.70
Mango	2.50	2.80	3.10	3.30	3.50	3.70	3.90	4.10

Table 2 Average reduction of concentration with temperature

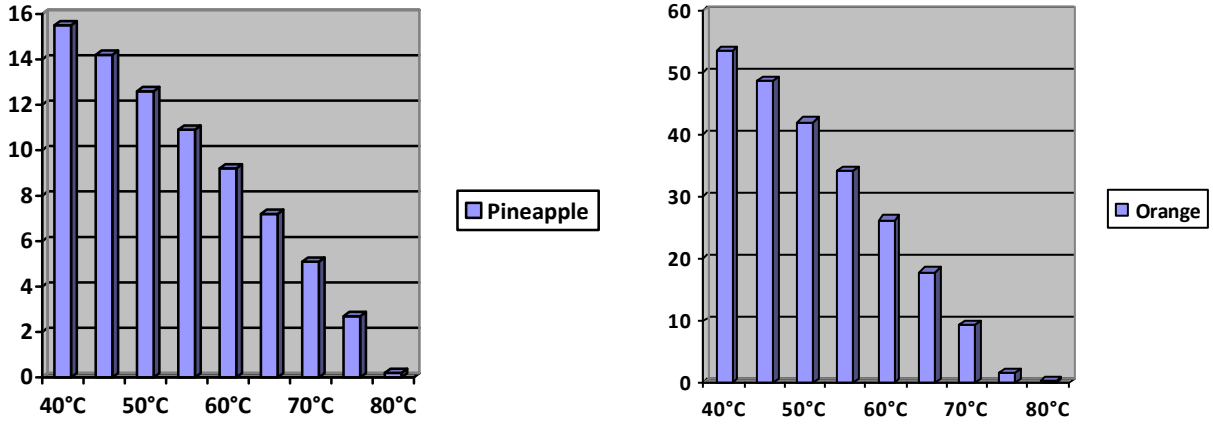

Fig. 1 Histogram showing the variation of Ascorbic acid with temperature for pineapple and orange.

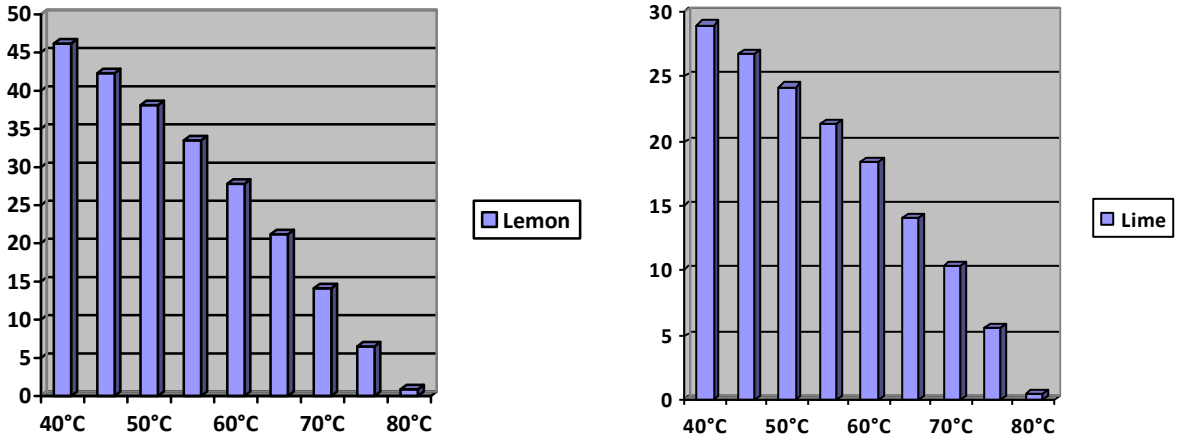

Fig. 2. Histogram showing the variation of Ascorbic acid with temperature for lemon and lime.

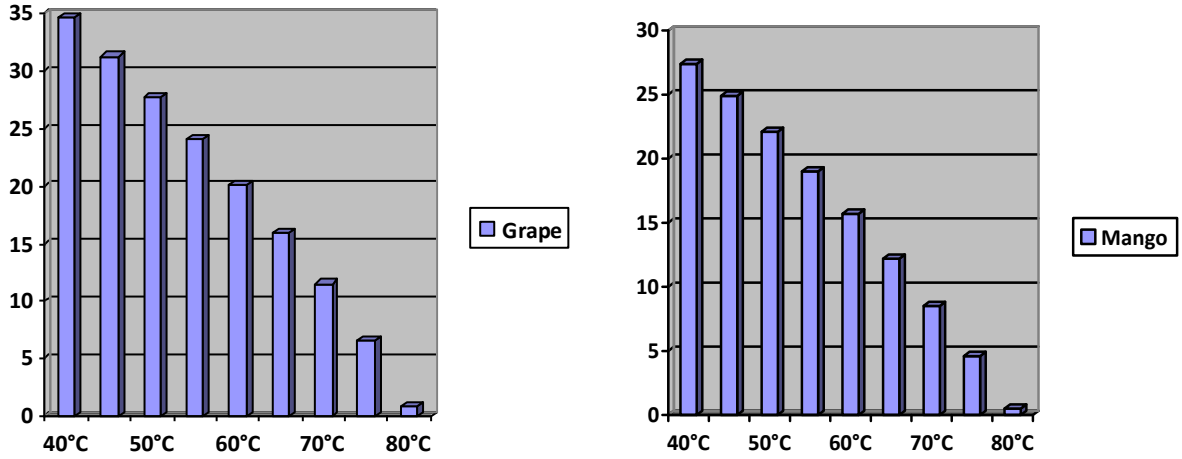

Fig. 3 . Histogram showing the variation of Ascorbic acid with temperature for grape and mango

Stability profiles of the various exotic tropical fruits

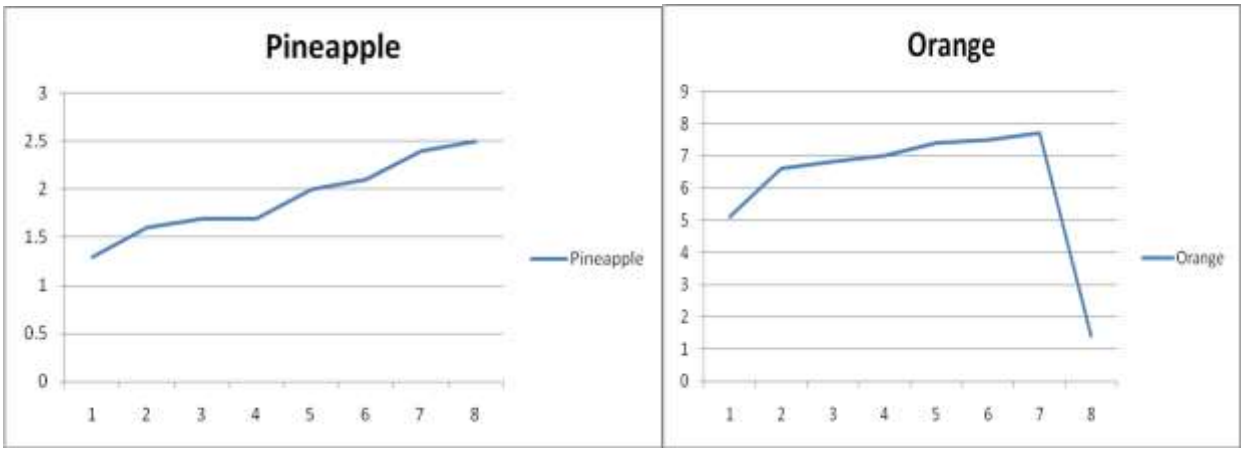

Fig. 4 . Stability profiles for grape and mango

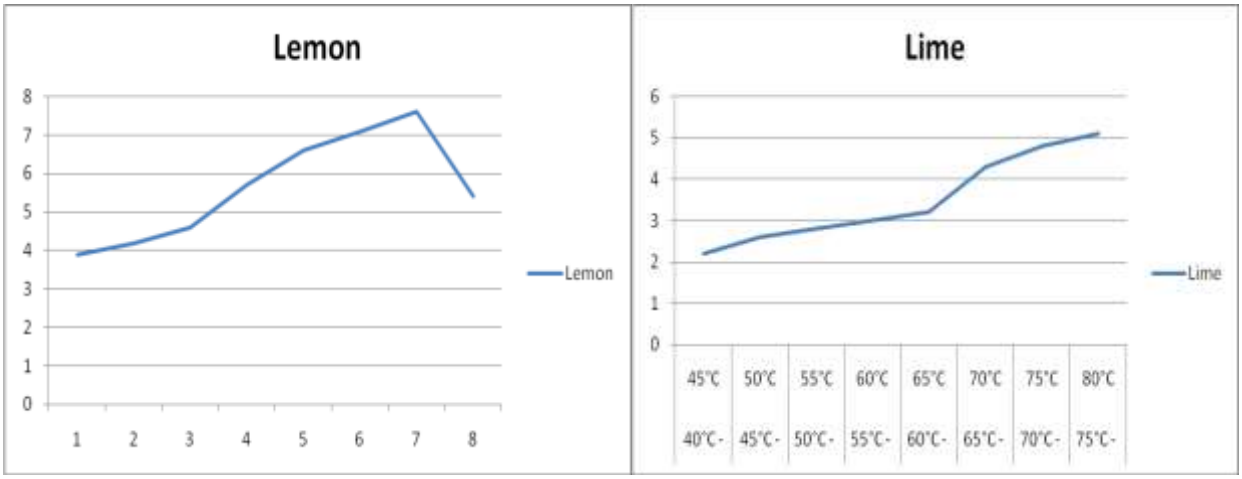

Fig. 5 . Stability profiles for grape and mango

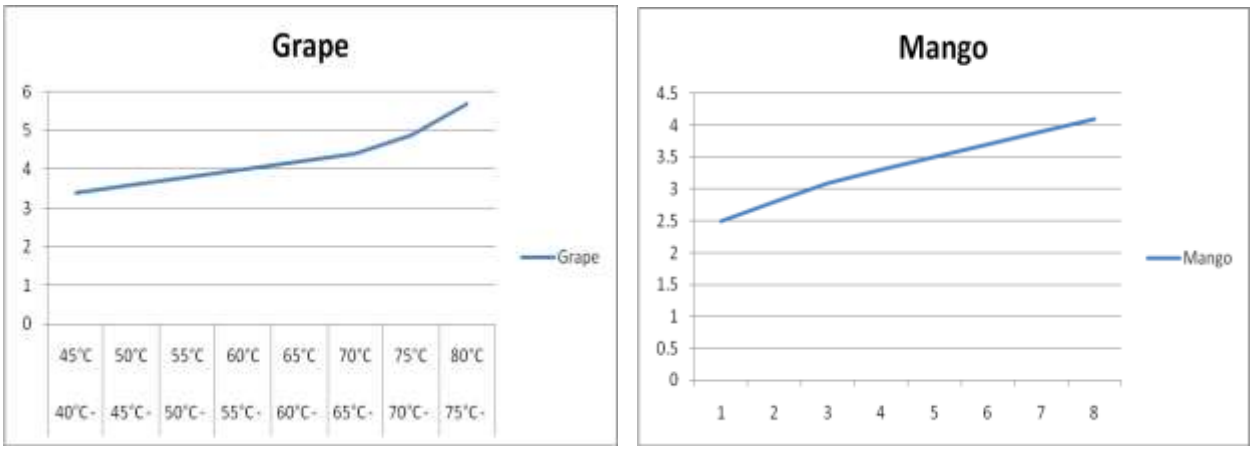

Fig. 6 . Stability profiles for grape and mango

DISCUSSION

The thermal stability of the ascorbic acid in some exotic tropical fruits like pineapple, orange, lemon, lime, grape and mango has been monitored using the dichlorophenolindophenol (DIPP) method and results show that the was a gradual decline of ascorbic acid content of these fruits with increase in temperature from 40°C to 80°C suggesting that the water soluble acid is heat sensitive. Pineapple declined from 15.50mg/100ml of extract at 40°C to 0.2mg/100ml at 80°C, orange declined from 53.70mg/100ml to 0.30 mg/100ml, lemon declined from 46.20mg/100ml to 0.90 mg/100ml, lime decline from 29.0mg/100ml to 0.50 mg/100ml whereas grape and mango declined from 34.70mg/100ml and 27.40mg/100ml to 0.90 mg/100ml and 0.50 mg/100ml respectively. The stability profile also showed that the rate of denaturation of this acid in the various fruits differ at each temperature as shown in their stability profiles indicating that the fruits have differing abilities to resist thermal induced degradation..from their stability profiles, orange and lemon showed a sharp drop 60°C whereas pineapple and lime showed similar behavior at all the temperatures. The profile of mango appeared almost linear while grape showed an increased stability beyond 60°C.

CONCLUSION

This result reaffirms the fact that traditional practices in the tropics does not permit storage of these fruits at elevated temperatures. The danaturation is suggested to be via the accelerated ring opening of the lactone ring thereby depriving the molecule of its antioxidant property as temperature increases. Nam, Nguyenb, Edward, Greenhalgha, Mohd, Kamaruddin, Jaouad, Kim, Georgios, Saamuel, John, Derek and Irvine, (2014) in their article "Understanding the acceleration in the ring opening of lactones delivered by microwave heating", reported the first detailed study focused upon the identifying the influence that microwave heating (MWH) has upon the mechanical step involved in the tin-catalyzed ring-opening of lactones such as caprolactones. It is possible that the lactone ring of the ascorbic acid which is the basic backbone of the molecule may have been ripped open as the temperature increased.

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