

## Studying the adulteration consequences of Guava Juice on vitamin - C loss at different packaging materials

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### ABSTRACT

This study focus on deterioration of vitamin - C and degradation of antioxidant activity of Guava juice during technological practice and caused by the adulteration of juice with sugars after diluted it to package juices. Different brands of commercial liquid Guava juice with different fabrics was included in this study. Furthermore, the effect of packaging materials on the stability of vitamin - C was investigated. Maximum vitamin - C content was observed in laboratory freshly Guava juice sample. Results revealed that processing applied to the Guava juice blends resulted to about 58 - 60% loss of ascorbic acid (AA). More effective factor on destroy vitamin - C was observed in brand samples with added sugar. The relatively high retained ascorbic acid values at 5°C was observed in brand samples without any additives while the lowest was found in brand samples with added sugar. Also, results appeared that the using of preservative material to increase the shelf life of vitamin - C or adding synthetic AA as nutritious compensation was ineffective in the presence of sweeteners of sugars within the processed juices. Untransparent glass bottles have been shown to effect ascorbic acid retention better than other packaging materials. On the other hand, brands of pure juice samples (without any additives) antioxidant activity will be totally depleted at maximum period of 255 days of storage at refrigerators (5°C) in glass bottles, but the minimum was observed in samples of plastic bottles which is 202 days.

**Key words:** Vitamin-C, Antioxidant activity, Packaging materials, Bioactive compounds, preservatives.

### Introduction

Fruits and their juice are becoming an important part of the modern diet in many communities. They are nutritious and plays a significant role in a healthy diet because they offer good taste and a variety of nutrients found naturally in them. Fruit juices are fat-free, nutrient-dense beverages, rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health and promote detoxification in the human body (Deanna and Bland, 2007).

Vitamin-C or ascorbic acid (AA) is a water - soluble vitamin that possesses diverse functions in the body and present in almost all fruits. It is an important antioxidant which functions as a co-substrate for many enzymes and helps to protect against several diseases. It has been used to prevent and control scurvy; but is also vital for many other important biological functions such as modulation of the immune system and control of inflammatory diseases, responsible in the synthesis and maintenance of collagen and neurotransmitters (Somchai *et al.*, 2016).

It can be interchangeable used as inhibitors of enzymatic browning (Baiano *et al.*, 2004). Humans do not synthesize vitamin-C due to the lack of L-gulonolactone oxidase in the final step of the vitamin-C synthesis, and thus it is important to acquire it from the diet. Also daily intake of vitamin-C is therefore needed because it cannot be stored in the human body (Aishah *et al.*, 2016). The consumption of ready - to - drink fruit juice has been growing at high rates, following the worldwide trend in consumption of healthy foods and the fast pace of life of the urban society (Boonpangrak *et al.*, 2016).

The trends in global fruit juice point to the tropical fruit markets that emerge as an attractive potential because of the diversity nutritional and therapeutic value due to its high content of natural antioxidant such as ascorbic acid.

Unfortunately, during the production of fruit juice, ascorbic acid may be lost due to the

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manufacturing process, which a thermal treatment during processing reduces the microbial load and inactivate enzyme present in the juice. At the same time, it promotes losses of vitamins, therefore, AA will often be fortified to compensate for the loss (Barros *et al.*, 2010).

Among the tropical fruits that have potential for agro-industrial use and thus participate in the composition of functional beverages, there is the Guava (Kaur *et al.*, 2011).

Guava (*Psidium guajava* L.) is the popular tropical fruits rich in vitamin-C and have the highest antioxidant capacities which may have potential beneficial effects on health. Its fruits contain five times more vitamin-C than citrus fruits, and ten times as pineapple fruits (Jawahear *et al.*, 2003). The Food and Drug Administration established for vitamin-C a Reference Daily intake equal to 60 mg and to 100 mg for smokers (FDA, 2005).

There are two problems for maintain vitamin-C content in the juices during processing, firstly, its sensitivity to oxygen, heat, light, alkali, and other factors such as packaging materials and storage condition. The second, emerging problem is the adulteration of juices with less expensive juices, colors, sugars, artificial sweeteners and other materials such as organic phosphate, calcium, sodium chloride, magnesium sulphate. All these reduces the stability of vitamin-C (Afshan *et al.*, 2015). Bacigalupi *et al.*, (2013) showed that orange juices had a loss of 53% vitamin-C after juice processing. While Boonpangrak *et al.*, (2016) showed that the vitamin-C degradation occur during the stages of Guava tropical juice processing (extraction, formulation, homogenization and pasteurization) was about 52% of its content, and found that after 4 days of storage in the refrigerator, approximately 15% losses of vitamin-C was observed.

Aishah *et al.*, (2017) investigated the deterioration of vitamin-C, lycopene, total phenolics, and antioxidant activity of ready – to – drink Guava juice. The results obtained showed the juice contain 39.79 mg/100mL of vitamin-C, 3.17 mg/L of lycopene, 28 mg/100mL of total phenolic content and 13.2 mMTE/100mL of ferric reducing antioxidant power, if Guava juice stored at elevated temperature after pasteurization.

There is little information related to the adulteration consequences of juices with less expensive juices, colors, sugars and sweeteners (Afshan *et al.*, 2015). For example, for the purpose of dilution of Guava drinks, sugars is added, which causes the breakdown of ascorbic acid, sugars invariably contains certain certain minerals that destroying the vitamin-C. The higher the sugar content the greater the loss of vitamin-C has been reported (Somchai, *et al.*, 2016). Therefore, vitamin-C is less in pasteurized juices than in fresh ones not only due to the thermal degradation of AA but also due to the addition of sugars, which dilute liquid juices.

Stability of vitamin-C can be maintained in an acidic environment or in the presence of other additives such as sodium nitrite or benzoic acid, other preservatives such as perchloric acid has been used to increase the shelf life of vitamin-C, but prolonged use of these preservatives is not without consequences. Oxidative properties of sodium nitrite are known for hepatic damage and several other organs (Salama *et al.*, 2013). No regulatory control has been imposed by the Government of countries for use of these preservatives in different juices. Manufacturers of the packed juices use any preservative, any amount at their free will causing public health hazard.

Similarly, the packaging has a direct effect on the availability of vitamin-C in juices. Various packaging materials such as high density polyethylene (HDPE), polypropylene (PP), metal cans, aluminium laminate and glass bottles are commonly used for packaging of fruit juices. Vitamin-C containing juices stored in metal or glass containers is stable, but when stored in plastic bottles, its shelf life decreases. On the other hand, metal cans are expensive and require sophisticated machinery for container closure (Chuan and Ku-Shang, 2006).

With innumerable fruits juice brands on local market and their manufacturers adulterate these products with different materials which commonly added to enrichment of the nutrient of fruit juice to get the desired value. At the same time reduces the stability of vitamin-C and became very low and do not reach the threshold value from it.

The focus of this work is to study the effect of these additives; sugars, preservatives, and fortified synthetic ascorbic acid on the degradation rate of natural vitamin-C and antioxidant activity during processing, packaging and cold storage.

Also; special attention that is given to evaluate the effect of different packaging materials on the vitamin-C retention in order to know how best and long Guava juice could be stored.

## Materials and Methods

All samples were collected to meet the requirement for analyzing the vitamin-C content from commercial juice samples. Different brands of commercial Guava juices were collected from the local market of Cairo, Egypt. Samples were stored at refrigerator for further analysis. All the packed samples were ensured to get analyzed 6 months before the marked expiry date on the pack.

### *Preparing freshly Guava juice:*

A total of 2 kg of mature and ripe fresh Guava fruit were plucked and gathered from an orchard at Giza. They were cutted and blended using a domestic blender (Ken wood chef classic, model KM 330). The blended Guava were then pressed to express the juice through double layer of cheese cloth. The obtained juice were filled into an untransparent glass bottle. To prevent oxidation of vitamin-C, 0.1% oxalic acid was added to sample.

### *Determination of vitamin-C:*

The high performance liquid chromatography (HPLC) method for analysis of ascorbic acid was modified from (Wimalasiri and Wills, 2010) was used. Briefly, 4 ml of sample was mixed with 4 ml methanol and 10 ml distilled water, filtered through 0.45  $\mu$ m membrane filter (Whatman International Ltd, Maidstone, UK). The first 3 ml of the filtrate was discarded and the next 1 ml was collected for analysis. 20  $\mu$ l of the sample was injecting into the HPLC system, column effluents were monitored at 254 nm, the UV absorbance maximum for ascorbic acid. The results were expressed in mg per 100 mL juice.

### *Determination of antioxidant activity:*

The antioxidant activity was measured according to Ferric Reducing Antioxidant power (FRAP) assay (Thaipong *et al.*, 2006) with the absorbance read at 593 nm. Standard curve was constructed against Trolox concentration (25 to 800  $\mu$ M) and results were expressed in mM TE/100mL of sample.

## Results and Discussion

Vitamin-C is an organic acid with antioxidant properties and one of the major nutritional components in Guava fruit. Since vitamin-C is easily destroyed during processing and because the adulteration of juices with sugars and other materials. Laboratory experiment result showed that the highest level of vitamin-C content is more evident in freshly Guava sample (233 mg/100mL), therefore, freshly Guava juice was thus demonstrated to be good sources of ascorbic acid with the highest value observed.

Vitamin-C content in commercially available Guava juice samples, collected from the local market at different packaging materials are tabulated in Table 1,2,3 and 4.

Results revealed that processing applied to the Guava fresh juice to prepare a pasteurized preservative juice (Nectar) with about 8-10% total soluble solids. It resulted to 58% loss of ascorbic acid. This decrease in vitamin-C may due to the oxidation and thermal degradation of ascorbic acid to dehydro-ascorbic acid (Mgaya-Kilima *et al.*, 2015). These losses were unavoidable due to the labile nature of vitamin-C as mentioned earlier.

The relatively high retained ascorbic acid values in the refrigeration temperature before 6 months of expiry date was observed in group 1 (Brands without any additives), while the lowest was found in brand samples with added sugar (group 2 , 5) which the loss percent was less than group one with about 48% (Table 1 to 4). This loss is due to sugar which invariably contains certain minerals that can cause breakdown of vitamin-C. This agrees with the report of Somchai *et al.*, (2016). They showed that orange juice had a loss of 53% after adding sugar to the packed juices.

Therefore, packed juices purchased from the local market, mostly, showed loss of vitamin-C during preservation process. For the purpose of dilution of juice drinks, sugar is added, which causes a

degradation of vitamin-C. This is one of the problems of juice adulteration consequences (Daglia *et al.*, 2000).

**Table 1:** Vitamin-C content (mg/100mL) in different brands of commercial Guava juices packed in glass bottles and stored at 5°C:

Brands	No. of samples	Range of AA values before 6 months of expiry date	Mean value	Range of AA values before 4 months of expiry date	Mean value	Losses (%)	Range of AA values before 2 months of expiry date	Mean value	Losses (%)
Group 1	3	89.20–95.50	92.33	82.07–87.86	84.96	7.98	77.56–83.03	80.26	5.53
Group 2	3	44.80–47.90	46.40	39.73–42.48	41.36	10.86	36.56–38.59	37.77	8.87
Group 3	3	58.88–63.30	60.89	56.24–58.71	57.33	5.84	54.60–56.49	55.53	3.14
Group 4	3	52.62–54.50	53.44	49.20–51.00	50.00	6.44	46.32–48.74	47.83	4.34
Group 5	3	40.40–51.21	45.70	37.57–48.10	42.56	6.87	35.33–46.00	40.58	4.65

Note: All collected brand samples had total soluble solids 8.8-10.0%

Group 1: Samples of brands without any additives (pure). Group 2: With sugar

Group 3: With preservative. Group 4: With sugar + preservative

Group 5: With sugar + preservative + Ascorbic acid.

**Table 2:** Vitamin-C content (mg/100mL) in different brands of commercial Guava juices packed in plastic packages and stored at 5°C:

Brands	No. of samples	Range of AA values before 6 months of expiry date	Mean value	Range of AA values before 4 months of expiry date	Mean value	Losses (%)	Range of AA values before 2 months of expiry date	Mean value	Losses (%)
Group 1	3	20.20–26.10	23.45	18.97–23.20	21.34	9.0	17.70–21.00	19.82	7.1
Group 2	3	14.70–19.27	12.19	7.78–13.38	10.91	10.5	6.72–10.64	8.96	9.4
Group 3	3	13.47–17.38	15.47	11.80–15.50	13.61	6.2	10.63–14.55	12.86	5.5
Group 4	3	10.85–14.87	12.89	10.22–13.71	11.98	7.0	9.90–12.80	11.17	6.8

Note 1: All collected brand samples had total soluble solids 8.8–10.0%

Note 2: Plastic packages samples are concluded high-density polyethylene (HDPE) & polypropylene (PP)

Group 1: Samples of brands without any additives (pure). Group 2: With sugar

Group 3: With preservative. Group 4: With sugar + preservative.

Note 3: Group 5 ; brands of sugar + preservative + Ascorbic acid in plastic bottles did not available within the local market.

Also results showed a degradation of vitamin-C during storage at refrigerator (Table 1 to 4). Jewaheer *et al.*, (2003) reported that the relative instability of vitamin-C prolonged storage may due to the presence of oxygen. Vitamin-C can easily be oxidized in the presence of oxygen by both enzymatic and non-enzymatic catalyst.

On the other hand, results appeared that the using of preservatives or adding AA was ineffective to increase the shelf life of vitamin-C in the presence of sweeteners and/or sugars within the processed juices (group 3, 4 and 5) which the proportion of vitamin-C retention was not evident in all samples. Therefore, only pure Guava samples (group 1) still contained the recommended daily allowance (RDA) for adults and smokers, which is 100mg.

Packaging is an important aspect in the juice processing industry as it serves the important functions of containing the product protecting the sterilized products against microbial contamination during their shelf life and protecting the nutritional status against chemical and physical damage while providing information on product features (Anin *et al.*, 2010). Various packaging materials such as glass, high-density polyethylene (HDPE), polypropylene (PP), aluminium and tetrapak cans are commonly used for packaging of fruit juice (March and Bugusu, 2007).

To illustrate this factor on vitamin-C losses, pure commercial Guava juice (without any additives, group 1) can give a true information about the effect of packaging materials individually on vitamin-C losses (Table 5 & fig 1).

Results revealed that vitamin-C losses was higher in the juice stored with plastic which vitamin-C decreased by fourfold than that stored with untransparent glass and about threefold than stored with tetrapak packages (fig 1). The vitamin-C content in the juice packed in glass bottles was

found to be 92.33, 84.96 and 80.26 mg/100mL at 6, 4 and 2 months before expiry date respectively while, in the juice packed in plastic bottles was 23.45, 21.34 and 19.82 mg/100mL at 6, 4 and 2 months before expiry date respectively. Therefore, polyethylene and/or polypropylene packaging materials was not as effective in preserving vitamin-C as the untransparent glass bottles. The later have been shown to effect ascorbic acid retention better than other packaging materials.

Anin *et al.*, (2010) showed that plastic containers contain a lesser level of plasticizers, these additives are usually have low molecular weight compounds possessing high mobility so, it often migrate from the polymer matrix by diffusion in a liquid medium and vitamin-C can easily be oxidized more faster. Also, Emelike and Ebere (2015) stated that vitamin-C losses was higher in plastic containers, this is because light might have penetrated it causing vitamin-C to leach out. Mgaya-kilima *et al.*, (2015) observed a higher decrease in the vitamin-C content of roselle-mango juice blends stored over a period of time from the first to the last month of cold storage at samples packed in polyethylene sachets and found that packaging material has a significant effect on the retention of vitamin-C of roselle-mango juice blends and suggested bottle packaging.

**Table 3:** Vitamin-C content (mg/100mL) in different brands of commercial Guava juices packed in tetrapak packages and stored at 5°C:

Brands	No. of samples	Range of AA values before 6 months of expiry date	Mean value	Range of AA values before 4 months of expiry date	Mean value	Losses (%)	Range of AA values before 2 months of expiry date	Mean value	Losses (%)
Group 1	3	74.51–79.83	77.18	67.20–73.90	70.87	8.20	63.53–69.22	66.29	6.46
Group 2	3	37.00–44.56	40.90	32.21–39.30	35.97	12.06	29.20–35.22	32.55	9.50
Group 3	3	42.18–50.10	46.46	41.23–47.00	44.41	4.40	39.91–45.30	43.07	3.00
Group 4	3	39.50–44.51	41.67	35.15–43.10	38.75	7.00	34.36–40.00	37.12	4.20
Group 5	3	35.05–39.10	37.04	32.41–37.46	34.61	6.56	29.45–36.46	33.12	4.31

Note: All collected brand samples had total soluble solids 8.8-10.0%

Group 1: Samples of brands without any additives (pure). Group 2: With sugar

Group 3: With preservative. Group 4: With sugar + preservative

Group 5: With sugar + preservative + Ascorbic acid.

**Table 4:** Vitamin-C content (mg/100mL) in different brands of commercial Guava juices packed in aluminium laminate packages and stored at 5°C:

Brands	No. of samples	Range of AA values before 6 months of expiry date	Mean value	Range of AA values before 4 months of expiry date	Mean value	Losses (%)	Range of AA values before 2 months of expiry date	Mean value	Losses (%)
Group 1	3	34.65–39.24	36.66	30.00–36.02	32.60	11.07	26.59–32.60	29.46	9.63
Group 2	3	13.50–19.48	16.39	10.22–17.10	14.24	13.11	9.88–15.20	12.60	11.51
Group 3	3	21.10–26.06	23.82	20.12–24.32	22.15	7.00	18.00–23.10	21.00	5.19
Group 4	3	17.00–20.71	19.06	13.23–17.20	15.61	9.50	11.78–16.44	14.43	7.52
Group 5	3	13.14–16.42	15.90	10.74–15.50	13.63	7.00	9.98–14.95	12.94	5.06

Note: All collected brand samples had total soluble solids 8.8-10.0%

Group 1: Samples of brands without any additives (pure). Group 2: With sugar

Group 3: With preservative. Group 4: With sugar + preservative

Group 5: With sugar + preservative + Ascorbic acid.

**Table 5:** Vitamin-C content (mg/100mL) of commercial pure Guava juice (100%) "group 1" packed in different packaging materials and stored at 5°C:

Packaging materials	No. of samples	Months before expiry date			
		* Zero	6	4	2
Glass	3	233	92.33	84.96	80.26
Tetrapak	3	233	77.18	70.87	66.29
Aluminium	3	233	36.66	32.60	29.46
Plastic	3	233	23.45	21.34	19.82

\*: Value of zero time were obtained from laboratory determination of antioxidant activity in freshly Guava juice sample.

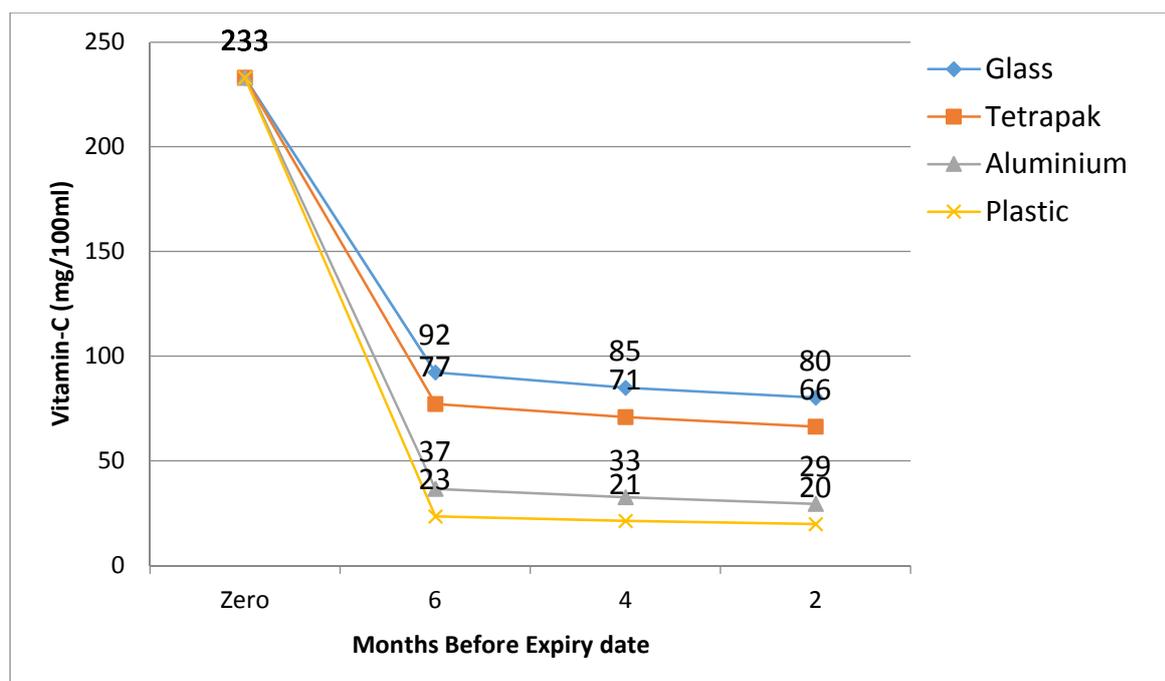


Fig. 1: Effect of processing & packaging materials on Vitamin-C of pure Guava juice (group 1) stored at 5°C

#### Degradation of antioxidant activity:

The defensive effects of natural antioxidants in fruits are related to three major groups: vitamins, phenolics and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidant while carotenoids are known as lipophilic antioxidant (Thaipong *et al.*, 2006).

In this study antioxidant activity determined as ferric reducing antioxidant power (FRAP) which measured both the hydrophilic and lipophilic antioxidant present in Guava juice. Table 6 & fig 2. represents the FRAP of Guava juice at different packaging materials of commercial pure juice (group 1) which stored at 5°C.

Results showed that FRAP of freshly Guava juice was 13.6 mMTE/100ml. this value was decreased to 10.6, 10.4, 10.2 and 9.7 after 60 days of cold storage in glass, tetrapak, aluminium and plastic packages respectively. After 200 days, the diminution of FRAP reached to 1.2 and 0.5 mM TE/100mL in glass and tetrapak packages respectively and to zero mM TE/100ml in aluminium and plastic packages.

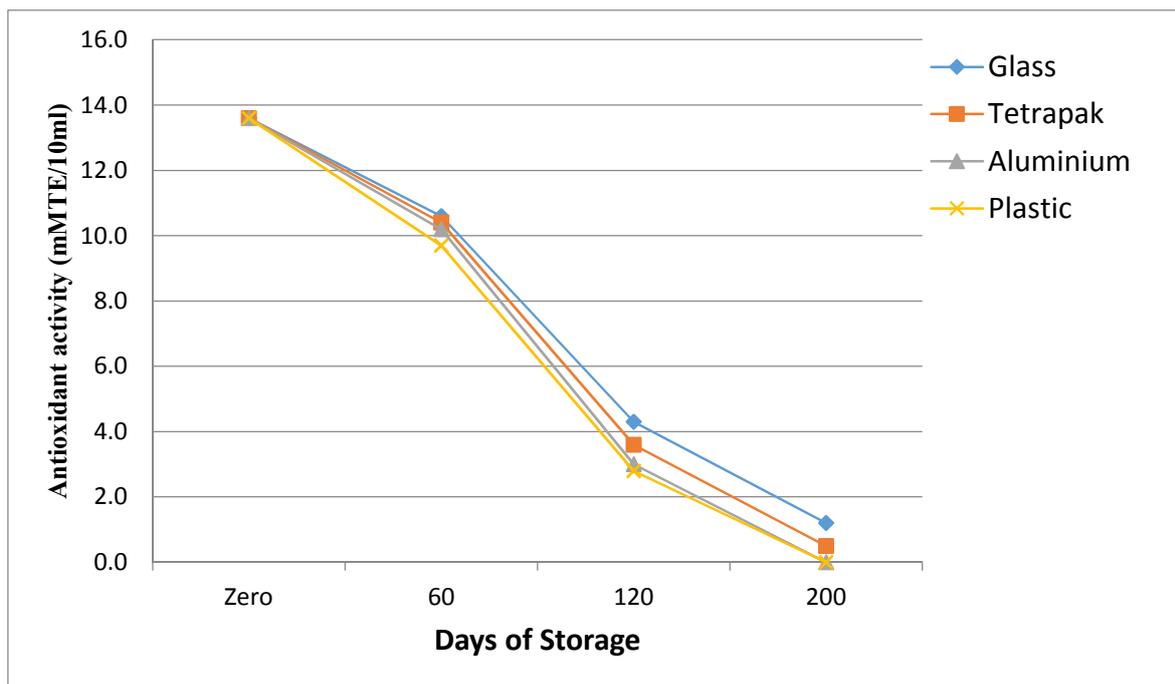
Based on the above values obtained, antioxidant activity will be totally depleted at 255, 243, 238 and 202 days of storage at refrigerated temperature of 5°C for glass, tetrapak, aluminium and plastic packages respectively.

Nearly, this observation is in agreement with the study of Aishah *et al.*, (2016). They determined the effect of storage temperature on antioxidant capacity in pink Guava juice and found that the freshly prepared ready-to-drink pink Guava juice, FRAP was totally depleted at 226 days of storage at refrigerated temperature. Pasupuleti and Kulkarni, (2013) revealed that a total depletion of the quality indicators in Guava juice were predicted at lower storage temperature, while, the faster loss of it at higher storage temperature.

Table 6: Antioxidant activity (mM TE/100mL) of commercial pure Guava juice (100%) "group 1" packed in different packaging materials and stored at 5°C:

Packaging materials	No. of samples	Storage / days			
		*Zero	60	120	200
Glass	3	13.6	10.6	4.3	1.2
Tetrapak	3	13.6	10.4	3.6	0.5
Aluminium	3	13.6	10.2	3.0	Zero
Plastic	3	13.6	9.7	2.8	Zero

\*: Value of zero time were obtained from laboratory determination of antioxidant activity in freshly Guava juice sample.



**Fig. 2:** Degradation of Ferric reducing antioxidant power of pure Guava juice (group 1) during storage at 5°C

Somchai *et al.*, (2017) stated that nutrient destruction is complex function of many variables such as pH, oxygen, presence of enzymes, metal catalysis, but the great variables are the presence of sugar and the kind of used packaging materials.

## Conclusion

Conclusion of this study is that the vitamin-C content of the commercial juices are very low and do not reach the threshold value of daily intake (60-100 mg) except samples without any additives (pure juice). On the other hand, when juices are treated by a preservative or fortified AA, the oxidation process is reduced but inefficiently due to the presence of sugars.

Further investigation should be carried out to find ways to stabilize these bioactive compounds during processing, packaging and storage in order for the consumers to obtain the maximum health promoting effects procided by fruit juice consumption throughout the shelf life of the juice, and juices do not be changed to serve as a cold drinks or beverages, uses for refreshment only.

Relatively, processed Guava juice can conveniently be produced and stored in untransparent glass bottles or tetrapak packaging for up to 6 months in refrigerator to retain the remain vitamin-C in the processed juice.

Antioxidant activity will be totally depleted at maximum period of 255 days of storage at 5°C in glass bottles, but the minimum was observed in plastic bottles which is 202 days.

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