COMPARATIVE ANALYSIS OF VITAMIN C CONTENT OF SOME LOCALLY AVAILABLE PACKED AND FRESH FRUIT JUICES BY REDOX TITRATION METHOD.

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Present study was conducted with the aim to compare the concentrations of vitamin C (ascorbic acid) in packed and fresh fruits, and to evaluate the potential benefits of fresh fruit juices over packed fruit juices. Fresh fruits such as mango, lemon, orange, apple (green) and apple (red) were purchased from supermarket in Farasan and brought to the biology laboratory of Faculty of Science and Arts in Farasan. The fruit samples were thoroughly washed with normal tap water, juice from each fruit was squeezed out manually without applying heat or any solvent, and filtered with a muslin cloth to get rid of pulp and seeds. However, the collected packed juices of the same fruits were simply unsealed and taken directly in a test tube for analysis. The juices thus extracted were then subsequently analyzed for ascorbic acid content of by iodometric titration method which is a simple and precise method. All the analyses were done in triplicates (n=3). The results of present study indicated that the concentration of ascorbic acid in the freshly collected juice samples was found to be: mango (88.10 mg/100 mL), lemon (49.32 mg/100 mL), orange (53.00 mg/100 mL), and apples (green, red) (29.10 and 28.3 mg/100 mL). However, the analyzed vitamin C content in packed juice samples of were 63.89, 47.51, 44.28, 25.30 and 11.90 mg/100mL, respectively for mango, lemon, orange, apple and pineapple juices. From these results it is concluded that to derive maximum benefits in terms of vitamin C content, the fruits must be consumed fresh.

Vitamin C is also known as ascorbic acid or L-ascorbic acid or antiscorbutic vitamin because deficiency of vitamin C causes scurvy which results in dermal hemorrhages of skin, inefficient and prolonged wound healing, edema and weakness. In ancient times, scurvy was common among the sailors who go on long voyage in the sea and thus do not get the dietary source of vitamin C as long as they travel in the sea.

Vitamin C is an essential nutrient that plays a vital role in protecting the body from infection and disease and is necessary in the synthesis of collagen in connective tissues, neurotransmitters, steroid hormones, carnitine, conversion of cholesterol to bile acid and enhances iron bio-availability (Robert et al., 2000). It participates in numerous
biochemical reactions, suggesting that vitamin C is important for bodily processes from bone formation to scar and tissue repair (Rickman et al., 2007), and also as an important anti-oxidant in the body.

Human body cannot synthesize this vitamin, therefore, it must be obtained through diet to fulfill the requirement. Most of the dietary vitamin C is taken by fruits and vegetables. Nearly all veggies are thoroughly cooked before being eaten, thus destroying the heat-sensitive vitamin C content. For this reason, vegetables cannot be trusted to supply the dietary vitamin C. Contrary to this, fruits are consumed raw, thus conserving the ascorbic acid content and hence guarantee adequate vitamin C nutrition. In view of the nutritional significance of vitamin C and for better utilization of fruits and vegetables as a source of this vitamin, clear understanding of their vitamin C content is necessary. Various factors such as regional varieties, climate and the method of estimation have pronounced influence on vitamin C content of fruits and its amount may be different even in the same fruits collected from different parts of the world.

In this study, the redox-titration method was adopted to analyze the vitamin C contents in the collected juice samples. This is a simple and authentic method that determines the vitamin C concentration in a sample solution by a redox titration involving iodine solution. The very idea behind this is that with the drop-wise addition of iodine in the sample during the titration, the ascorbic acid present in the sample starts being converted to dehydroascorbic acid, whereas the iodine is reduced to colourless iodide ions which do not react with starch to produce coloured complex.

When all the ascorbic acid of the sample gets oxidized then additional iodine added in the sample from the burette will be as molecular iodine which on combining with the starch indicator solution present in the sample, changes the colour of the solution which is the indicator that now the end point has been achieved. This approach has also been used by various other worker in the past, some with slight modification and some with almost the same (Tee et al., 1988, Okiei et al., 2009, Katz 2013, Tareen et al., 2013). Data on vitamin C content of fruits are available for various other countries and regions (Aydogomus et al., 2002, Melo et al., 2006, Wall et al., 2006, Rickman et al., 2007, Okiei et al., 2009, Nour et al., 2010, Aurelia et al., 2011, Tiriwork & Ghirma 2012; Nweze et al., 2015, Tareen et al., 2015). However, this information is absolutely lacking for Farasan Island, near Jazan province, Kingdom of Saudi Arabia.

In view of this, present research work was conducted to determine the vitamin C contents of the locally available packed and the fresh fruit juices so that health conscious people may be more benefited with this.

Materials and Methods:-

Procurement of the sample:-
Packed juice and the fresh fruit samples as have been listed in Table 1, were purchased from the supermarket in Farasan and brought to the Biology Laboratory of Faculty of Science and Arts in Farasan. The choice of the juices and fruits were based on their availability and cost-effectiveness. Since, fresh pineapple fruit was not available in the market, it was not included in the study.

Sample Preparation:-
The collected fruits were washed thoroughly with normal tap water and the juice was extracted by manual squeezing of the fruits. Mango, lemon, orange, and apple (red and green varieties) juices were prepared fresh. The juice samples were then filtered using a muslin cloth to remove pulp and seeds, and transferred in already labeled test-tubes. However, the packed juices were unsealed and then subjected to vitamin C analysis. The study was carried out in Biology laboratory of Faculty of Science and Art in Farasan.

Analysis of Vitamin C Content of the juice samples:-
Vitamin C content of the packed and fresh fruit juice samples was determined by iodometric titration method. The reagents required are listed as under:

a. Vitamin C standard solution
b. Standard iodide solution (KI)
c. Starch indicator solution
d. Deionized water

Preparation of Reagents:-
Vitamin C standard solution:-
To prepare this solution, 10mg vitamin C was dissolved in 10 mL water. This represents a concentration of 1mg vitamin C/mL.
Standard iodine solution:-
To prepare this, 10g reagent grade KI is carefully weighed on the electronic balance (Carolina, www.carolina.com). The content is carefully dissolved in a known amount of water, then few iodine balls were added and raised to the marked volume of 100 mL with distilled water.

Starch indicator solution:-
It is prepared fresh by dissolving 1 g starch in 100 mL water. To prepare this, 1g starch powder is added into 10 mL distilled water, stirred well and then transferred to a 100 mL boiling water. The content is stirred thoroughly and boiled for a minute and then allowed to cool at room temperature. It will lead to the formation of precipitate once it becomes cool. Decant the supernatant and use it as the indicator solution. However due to unavailability of starch powder, rice powder was used to make the above reagent in this study.

Procedure of vitamin C Analysis:-
In a 7 mL juice sample, 2 mL of starch indicator solution was added. The resulting solution was then titrated with the drop-wise addition of standard iodine solution near the end point. Following reaction occurs during this:

\[
\text{Ascorbic acid (C}_{6}\text{H}_{8}\text{O}_{6}) + \text{I}_2 \rightarrow 2\text{I}+ \text{Dehydroascorbic acid (C}_{6}\text{H}_{6}\text{O}_{6})
\]

(Reduced form) (Oxidized form)

Calculations:-
The mL of iodine solution used in the titration of standard vitamin C solution and that of the juice samples are then noted down. The vitamin C contents of the samples were calculated in terms of the mL of titrant (standard iodine solution) used for standard vitamin C solution and for the juice samples. 12 mL of standard iodine solution reduces 10 mg vitamin C in standard vitamin solution. This is a fixed value. Using this value, the amount of vitamin C is easily calculated for every unknown juice samples as per following calculation:

\[
\frac{\text{mL of iodine solution used for vitamin C standard solution}}{10 \text{ mg vitamin C}} = \frac{\text{mL of iodine solution used for juice samples}}{X \text{ mg vitamin C}}
\]

where X=unknown and is the amount of vitamin C in mg as estimated for the juice samples after calculation using the above formula.
Statistical Analyses:
All the analyses were done in triplicates (n=3). Statistical evaluation and graphical representation of the data were done using MS Excel (Office 2008).

Table 1: List of the collected fresh fruit samples.

| Fresh fruits       | Scientific names         |
|--------------------|--------------------------|
| Mango              | Mangifera indica         |
| Lemon              | Citrus limon             |
| Orange             | Citrus nobilis           |
| Apple (red)        | Mallus domestica         |
| Apple (green)      | Mallus domestica         |
| Pineapple          | Ananas comosus           |

List of packed fruit juice samples

| Brand | Mango | Lemon | Orange | Apple | Pineapple |
|-------|-------|-------|--------|-------|-----------|
| Nadec |       | Nadec | Nadec  | Nadec | Ceaser    |

Table 2: Amount of vitamin C in the collected samples.

| Samples | In. reading of burette | Fn reading of burette | volumes of iodine solution used | mg of vitamin C/100 mL |
|---------|------------------------|-----------------------|---------------------------------|------------------------|
| Mango   | 25.00                  | 19.63                 | 5.40                            | 63.89                  |
| Mango   | 25.00                  | 17.60                 | 7.40                            | 88.10                  |
| Lemon   | 25.00                  | 21.10                 | 3.90                            | 47.51                  |
| Lemon   | 25.00                  | 20.9                  | 4.10                            | 49.32                  |
| Orange  | 25.00                  | 21.30                 | 3.70                            | 44.28                  |
| Orange  | 25.00                  | 20.50                 | 4.50                            | 53.00                  |
| Apple   | 25.00                  | 22.90                 | 2.10                            | 21.30                  |
| Apple   | 25.00                  | 22.59                 | 2.41                            | 29.10                  |
| Apple   | 25.00                  | 22.56                 | 2.44                            | 28.30                  |
| Pineapple | 25.00               | 24.00                 | 1.00                            | 11.90                  |

Comparison of vitamin C Concentration in Packed and Fresh Fruit Juices

| Fresh fruits          | Average vitamin C concentrations (mg /100mL) |
|-----------------------|---------------------------------------------|
|                       | Packed juice (labeled value) | Packed juice (analyzed value) | Fresh fruit juice (analyzed value) |
| Mango                 | 110                          | 63.89                         | 88.10                          |
| *Lemon                | 45                           | 47.51                         | 49.32                          |
| Orange                | 25.00                        | 44.28                         | 53.00                          |
| Apple (green)         | 25.00                        | 21.30                         | 29.10                          |
| Apple (red)           | 25.00                        | 21.10                         | 28.30                          |
| Pineapple             | 15.20                        | 11.90                         | NA                             |

*=packed juices, *=fresh fruit juices, *mL, In=initial, Fn=final, *= information related to vitamin C for lemon juice was not mentioned on the pack.
Results:
In present study, packed juices (Nadec and Ceasar Company) and fresh fruit juice samples were analyzed for their vitamin C contents. The results for this analysis are tabulated in the Table 2. The analyses revealed significant differences in the vitamin C contents of analyzed packed and fresh juice samples. Data for this have been tabulated in Table 2. The vitamin C content of fresh lemon and orange juice were found to be almost similar (P>0.05), while that of green apple juice was significantly lower than these two (P<0.05) followed by the red apple. However, the vitamin C contents of fresh apple and packed pineapple juices showed slight difference (P>0.05). Excepting for packed sample of mango juice in which the vitamin C was analyzed to be 63.89 mg/100 mL in contrast to the labeled value of 110 mg/100 mL, the trend for the analyzed vitamin C contents of other packed fruit juices was almost similar to their labeled values.

For more clear understanding, the results are also presented graphically in Figure 1.

Discussion:
The results summarized in Table 3 indicate that each juice sample has a different titre value to attain its end point. This is due to the differences of the amount of vitamin C in different fruits. The juice with lower amount of vitamin C required less of the titrant to attain its end point whereas the juices with higher amounts of vitamin C required more of the titrant to attain the permanent end point-colour. This is due to the fact that the colour changes when all the vitamin C of the sample has been completely reduced and only starch is in the solution which combining with the iodine from the titrant produces purplish-blue colour. The results of the freshly squeezed fruit juices showed significant difference with the packed product of the same juice.
In this study, highest value for analyzed vitamin C was found in mango juice, at 63.89 mg/100 mL for packed mango juice and 88.10 mg/100 mL for freshly squeezed mango juice, respectively. Lemon and orange juices had 47.51 and 44.3 mg/100 mL analyzed vitamin C content in packed juice samples, and 49.32 and 53 mg/100 mL in fresh juices. However, the lowest analyzed values for vitamin C as 11.9 mg/100 mL were recorded for packed pineapple juice in contrast to the labeled value as 15.2 mg/100 mL. The results of this study are within the range reported by various other workers in the past. Aydogmus et al. (2002) determined vitamin C content of orange juice obtained by squeezing in the range of 33 to 50 mg/100 mL. Melo et al. (2006) reported the value 37.34 mg/100 mL for orange juice. Okiei et al. (2009) reported that the ascorbic acid content of freshly prepared lemon juice is 48.61 mg/100 mL, respectively. Aurelia et al. (2011) reported the ascorbic acid content of lemon juice 54.74 mg/100 mL and Orange juice 39.25 mg/100 mL. Tiruwork and Ghiro (2012), reported the ascorbic acid content of freshly prepared orange juice and old orange juice were 41.4 mg/100 mL and 32.4 mg/100 mL, respectively. Ndefie et al. (2013) reported the vitamin C content of different brands of the packed juices between 27.28 to 37.14 mg/100 mL. Nour et al. (2010), reported that the average ascorbic acid was highest in lemon juice followed by sweet orange juice. However, Bekele and Geleta (2015) have reported highest vitamin C in mango juice than in lemon and orange juices. Highest vitamin C content as 110 mg/100 mL has also been reported for packed mango juice by Nadec in this study. However, in this study, the analyzed value was slightly higher than the labeled value of vitamin C for packed mango juice. The slight differences obtained by different workers mentioned above and that in the present study may be due to differences in the methodologies, method of juice extraction, different brands of packed juices, different storage temperatures and also due to differences in the climatic conditions. Despite all these factors, the analyzed vitamin C contents of the fruit juices in this study fall within the range reported by the above workers and do not show wide differences.

It was known that fresh fruit juices normally contain more vitamin C compared to packed juices. The results of this study also prove this. Very often the packed juices are not recommended to patients such as the kidney patients as they have some fruit stabilizers which are not good for the health of such people. Also, in case of sugar patients, it not advisable to drink more of the packed fruit juices as these juices often have adequate amounts of added sugar which may affect their health adversely. It is also known that fresh fruit juices have hundreds of times more nutrients, enzymes, and phytochemicals which are generally destroyed during process of their packaging.

The result of this study also showed that all of the fresh fruit juices analyzed, contained significantly higher vitamin C concentrations compared to those found in packed juices for the same fruits.

Conclusions and Recommendations:-

The results of the present research work clearly indicates that to derive maximum benefits in terms of vitamin C content, the fruits must be consumed fresh. It also supports the common perception that fresh is the best.

It is, therefore, recommended that nutrition and health education is needed to promote increased consumption of fresh fruits. Also attention should be given on the issue that from the varieties of fruits available in the market, which should be consumed to gain maximum vitamin C intake. Furthermore nutrition labeling of the fruits in terms of vitamin C content should be done.

This will do a lot to promote consumer awareness and the nutritional status of the populace to avoid deficiency diseases of vitamin C. The results of this study are very beneficial to stay hale and hearty in daily life.

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