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Research Article

Quantitative Determination of Ascorbic Acid in Commercial Fruit Juices by Redox Titration

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ABSTRACT
A number of commercial juices of different brands are available in the markets. These juices are consumed daily by all classes of population all over the world. In the present study ascorbic acid (AA) contents of 13 commercial juices of different brands in local market of Gilgit-Baltistan were quantitatively determined. The procedure is based on iodometric titration where dark blue complex forms in the presence of starch at the end point. The ascorbic acid content of All Pure juices produced by Shezan™ were found to contain highest concentration of ascorbic acid for mango, grape, orange, and pomegranate (53.54, 49.12, 47.58, and 41.13 mg/200 mL, respectively) whereas Elexire, Fruit Edge, and Slice produced by Rani™ contained the least quantity of ascorbic acid (7.74, 7.09 and 3.87 mg/200 mL, respectively). The comparative analysis also revealed that most of the commercial juices do not mention the AA data while others are misleading, although the amount of AA contents changes with the increased shelf life.

Key Words: Ascorbic acid, iodometric titration, commercial juices

INTRODUCTION

Vitamins are vital and very important to the survival and smooth functioning of human bodies. They are not produced or stored in the human body, therefore, the supply of vitamin C on the regular bases are essential and crucial for healthy body. Vitamins don't provide any direct energy but they function in combination with other substances in the body to supply energy (Osunde, *et al*. 2007, Karla, 1998, Arayne, *et al*. 2009). Vitamins are also required only in a very small quantity to maintain good health. Around thirteen different vitamins including eight B vitamins are essential that are required to our body on daily bases. The recommended daily intake of ascorbic acid is 60 mg in aggregate (Sarkiyayi, *et al*. 2010, Ogbuanu, *et al*. 2014).

Ascorbic acid (AA) also known as vitamin C can be obtained from fruits or vegetables and multi-vitamin supplements. Ascorbic acid is important to the body for many reasons. It helps to regulate blood pressure, reduction of cholestrole level, scavenging of free radicals which are associated to many complications like acceleration in aging (Zaman, *et al*. 2013, Zeerati, *et al*. 2014, Harrison, *et al*. 2014, Mitra, *et al*., 2014). Vitamin C intake may be particularly helpful to smokers, as they are more likely to suffer from oxidative stress and cell damage that can deplete AA. It also involve in prevention of scurvy. Scurvy is a disease which can affect muscles weakness, swollen and bleeding of gums, loss of teeth and bleeding under the skin as well as tiredness and depression (Dumbrava, *et al*. 2012, Pisoschi, *et al*. 2011, Roman, *et al*. 2013).

Several analytical methods have been reported for the determination of AA using titrimetry (Igwe, 2014, Highet, *et al*., 1942, Farooqi, *et al*.1990), spectrometry (Janghel, *et al*. 2012, Gmus, *et al*. 2002, Tahirovic, *et al*. 2012, Anwar, *et al*. 1990, Hussain, *et al*. 2010), HPLC (Mitic, *et al*. 2011, Khosravi, *et al*. 2014, Nojavan, *et al*. 2008, Shintani, 2013, Tee, *et al*. 1988), and amperometry (Wonsawat, 2014, Kumar, *et al*. 2013). One of the common techniques used for the quantitative determination of various nutrients is the titration method (). The amount of AA in any unknown sample can be determined with the help of iodometric titration. In this method, iodine reacts with ascorbic acid to produce dehydroascorbic acid which is a colourless product. Iodine also reacts with starch to produce a dark blue product (Figure 1).

In presence of both ascorbic acid and starch, the iodine preferably reacts with the Vitamin C instead of the starch. Therefore, when iodine is added in the presence of both substances, the ascorbic acid reacts and the products are colorless. When the AA has been completely used up, the iodine then reacts with the starch, and a dark blue product is formed. Hence, by the addition of iodine in drop wise manner, all of the Vitamin C utilizes first and the solution turns dark blue in the end.

A number of different brands are producing many types of fruit juices for the consumption of all classes of the population all over the world. It was noticed that most of the commercial juices available in the local market of Gilgit-Baltistan were found without any reported data for ascorbic acid contents on the box. In addition to this, all the commercial products available in this remote area of

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Table 1: Data of reported and measured ascorbic acid contents in commercial juices

S. No	Type of Juice	Brand Name	Trade Name	Vitamin C Value (mg/100 ml)	
				Reported	Observed
1	Mango	Rani	Fruit Edge	*NA**	07.09±1.37
2			Elexire	*NA**	07.74±2.12
3			Slice	*NA**	03.87±2.64
4		Shazan	All Pure	33.60	53.54±1.04
5			Mango Fruit Drink	24.00	16.80±1.93
6		Nestle	Nesfruita	*NA**	11.71±2.16
7			Chaunsa Nectar	16.00	13.55±1.71
8	Apple	Fruiticana	Apple Juice Drink	40.00	33.50±1.82
9		Nestle	Nesfruita	*NA**	9.31±2.05
10	Grape		Fruita Vitals	20.00	14.85±3.10
11		Shazan	All Pure	33.60	49.12±1.52
12	Orange		All Pure	33.60	47.58±1.47
13	Pomegranate		All Pure	28.00	41.13±2.34

**Data not available*

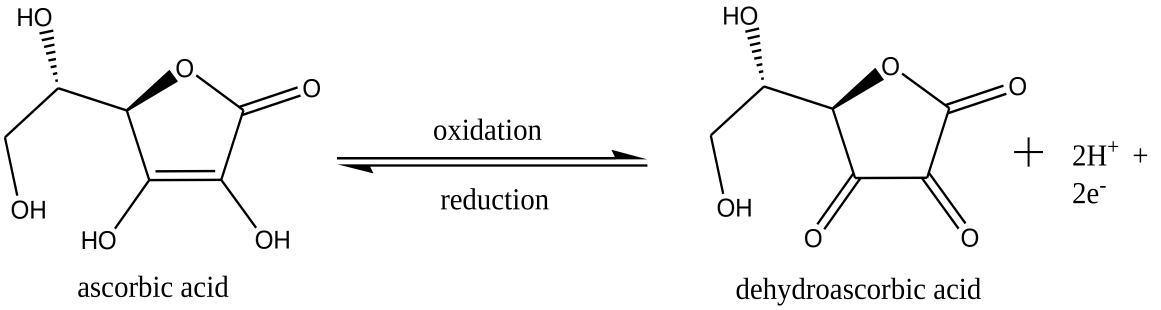

Fig. 1: Conversion of ascorbic acid to dehydroascorbic acid in the presence of oxidizing agent

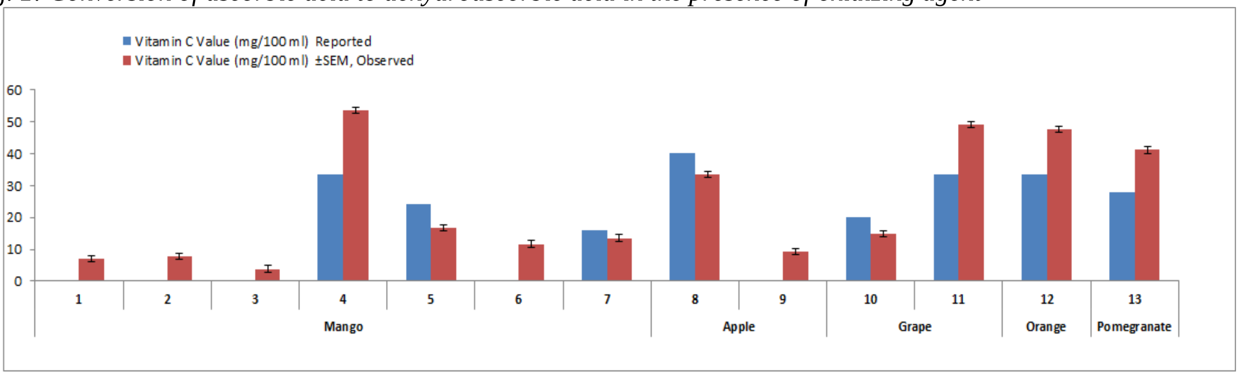

Fig. 2: Difference in dictated and actual amount of ascorbic acid of some commercial juices

Pakistan comes from very far big cities of the country after crossing tens of hundreds kilometers of distance. In this way it takes several days even over a week on transportation. This motivated us to investigate the issue to measure the AA contents of both type of juices with and without reported/dictated data on the labels. The finds are indeed very useful and beneficial to local communities to ensure the balance diet in their life routine.

Experimental Procedure

Preparation of 0.005 mol L^{-1} iodine solution: Accurately weight 2 g of KI and 1.3 g of I_2, dissolved in few mL of water and shake until dissolving. Transfer iodine solution to a 1 L volumetric flask, making sure to rinse all traces of solution in to the volumetric flask using distilled water, completed the volume up to the mark.

Preparation of 0.5% starch indicator solution: Soluble starch (0.25gm) to a 100 mL conical flask and 50 mL of distilled water was added. Solution heated with stirring at 80 ^{0}C for 5 minutes, ensuring not to exceed the stated temperature. Allow solution to cool to room temperature.

Preparation of drink sample: Industrial Packaged fruit juices of different suppliers Mango (Rani, Shazan, and Nestle), Apple (Fruiticana and Nestle), Grapes (Shazan and Nestle), Orange (Shazan), and Pomegranate (Shazan) were strained through cheese cloth where it contains a lot of pulp or seeds.

Standardization of the iodine solution with the Ascorbic Acid standard solution: Iodine solution changes rapidly because I_2 is a halogen gas that evaporates quickly. Therefore, iodine solutions need to be standardized all the time during the experimental procedure. Ascorbic acid is also susceptible to oxidation by atmospheric oxygen over time. For this reason, the sample was prepared immediately before the titrations. In addition to this, a small amount of oxalic acid (2 mL) was added to standard

Calculations

$$\text{mg of vitamin C in 1 mL of solution} = \frac{\text{mg of vitamin C (standard value)}}{100\,\text{mL}} \times 1\,\text{mL}$$

$$\text{mg of vitamin C oxidized by 1 mL of iodine reagent} = \frac{\text{mg of vitamin C in flask}}{\text{Average volume (mL) of iodine reagent}}$$

$$\text{mg of vitamin C in juice sample} = 20\,\text{mL} \times \frac{\text{mg vitamin C}}{1\,\text{mL of drink}}$$

ascorbic acid solution in order to minimize the probability of oxidation of ascorbic acid.

Titration

- Aliquot of the sample (20 ml) solution prepared above transferred into a 250 mL conical flask, 2 ml of oxalic acid, about 150 mL of distilled water and 1 mL of starch indicator solution.
- Samples were titrated with 0.005 $molL^{-1}$ iodine solution. The endpoint of the titration was identified as the first distinct trace of a dark blue-black color due to the formation of starch-iodine complex.
- Titration were repeated with further aliquots of sample solution until concordant results (titres agreeing within 0.1 mL) were obtained.

RESULTS AND DISCUSSION

Ascorbic acid is an important vitamin for human health and also acts as co-factor for many enzymes. It is therefore needed for physiological functions in the body especially for the synthesis of important substances including collagen, and some neurotransmitters. Ascorbic acid accelerates hydroxylation reactions by increasing the activity of enzymes hydroxylase and oxygenase.

Present study is focused to know whether the amount of vitamin C shown on boxes was equal to the real amount in the commercial fruit juices. In order to answer this question the ascorbic acid contents of thirteen commercial juices of three different brand names sold with prescription (Shazan, Nestle, and Fruticana) and two well-known brand names sold in the market without prescription for ascorbic acid (Rani and Nestle) were evaluated. The ascorbic acid levels in the juices were measured by using iodometric titration method. Table 1 show the brand names, type of fruit juice, trade name, and amount of vitamin C shown on the box as well as the actual amount found during present investigation. Amount of vitamin C in each juice were analyzed using iodometric titration method as explained in experimental section. The results of each measurement were shown in table 1 and figure 2. Every analysis was performed in triplicate where deviation was found bellow 5% which means all the measurements and test results are reliable. The table also shows the calculated and given ascorbic acid amounts in commercial juices with respect to amounts of titrated iodine solution (ml) and volume of sample fruit juice. Four of them were found to include less vitamin C than the amount shown on the box (Shazan, Nestle, and Fruiticana). The remaining four producing by Shazan were found to include more than the amounts shown on the box. However, the ascorbic acid contents of three fruit juices produced by Rani and two by the Nestle without any prescription on the boxes showed a very low amount of ascorbic acid.

The highest amount of vitamin C amount among all commercial juices were mango, grapes, orange and pomegranate produced by Shazan with trade name of All Pure, following apple juice of Fruiticana and grape juice by Nestle (Figure 2). The decreased amount of AA acid in some juices may be due to long shelf life, expose to high temperature and self-oxidation of ascorbic acid. But it is surprising that the All Pure fruit juices produced by Shazan showed higher AA contents compared to the dictated record on the boxes labels. None of the commercial fruit juice was found to have the same AA contents as mentioned on the boxes while many of the brands are marketing these products without prescription of vitamin C. In the conclusion the labels on commercial juices are misleading and don't represent the quantity of actual AA contents. These deviations might result from many different conditions which require further investigation.

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