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ASCORBIC ACIDS CONTENTS IN COMMERCIAL JUICES AND BEVERAGES

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Vitamin C is water soluble which is essential for many physiological processes. Humans cannot synthesize vitamin C, so it must be taken through diet or as food supplement. Nowadays many liquid juices available in the market claim high levels of vitamin C. The present study was designed to check levels of vitamin C in commercially available juices and to verify manufacturers claim of vitamin C addition. Twenty five (25) juices, beverages, solid soft drinks of various brands were purchased from five locations in Islamabad and their ascorbic acid content was determined by Polarography. Juices were categorized into four groups namely international companies, Pakistani companies of good repute, other local companies, and solids drinks/ vitamin C supplement tablets. Results showed that measured and reported values of vitamin C in some international brand juices are comparable while others are not. Most of the reputed companies of Pakistan origin did not have any vitamin C in their products or their amount was negligible when compared with the reported values. Overall vitamin C was below detectable limit in juice packs of local companies. Vitamin C concentration in vitamin C tablets and solid soft drinks is comparable with reported values. On the basis of this study it is suggested that local companies may be using some chemical to develop taste and flavor of vitamin C in their products. It is recommended that strict quality control be implemented at the factories to prepare such products with correct amounts of vitamin C.

Keywords: Ascorbic acid, Juices, Vitamin C, Flavor, pH

1. Introduction

Vitamins are a class of nutrients that are required by the body for its various biochemical and physiological functions. Vitamins are subdivided into fat-soluble and water soluble vitamins. Fat-soluble vitamins are those that are soluble in organic solvents. These are A, D, E and K. Water soluble vitamins are vitamin C and B series that are usually termed as vitamin B complex. They can be easily dissolved in water. Human body cannot synthesize vitamin C, requiring its ingestion from exogenous supplements or diet. It has been reported that the cause of human inability to synthesize ascorbic acid is the absence of the active enzyme, l-gulonolactone oxidase from the liver [1]. Likewise, infections deplete the body stores of vitamin C, thus making the body immune system weak.

Vitamin C is an antioxidant and reducing agent. A reducing agent is a substance which helps in reduction, but itself undergoes oxidization. Vitamin C also acts as an antioxidant and protects the body from the deleterious effects of free radicals, pollutants and Reactive Oxygen Species (ROS). It also contributes to the synthesis of amino acid carnitine and the catecholamine's that regulate the nervous system. It also helps the body to absorb iron and breaks down histamine, the inflammatory component of many allergic reactions [2]. Vitamin C helps in the metabolism of tyrosine, folic acid and tryptophan. It not only helps to lower blood cholesterol but is also needed for tissue growth and wound healing. Vitamin C regulates the formation of neurotransmitters and increases the absorption of iron in the gut. Vitamin C is available either in natural or synthetic form. Tablets, capsules, and chewable are probably the most popular forms of vitamin C supplements, but vitamin C also comes in powdered crystalline, effervescent, and liquid forms. It is available in reduced form (L-ascorbic acid) and oxidized form L-dehydroascorbic acid [3].

The recommended daily intake of vitamin C varies according to age, sex, risk group (cigarette

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smokers, alcohol users, institutionalized elderly and subjects on certain drugs) and criteria applied in individual countries. The recommended dietary allowances (RDA) for vitamin C in the USA were recently revised upwards, from 60 to 90 mg/day for men and 75 mg/day for women based on pharmacokinetic data. For smokers, these recommended dietary allowances for vitamin C are increased by an additional 35 mg/day, for pregnant woman 85 mg/day and lactating women 120 mg/day [4]. The RDAs are in a similar range in other countries. Recent evidence sets the estimate for the need to maintain optimal health in the region of 100 mg daily. The current recommended daily acceptance (RDA) for ascorbic acid is suggested to be more than existing levels to achieve cellular saturation and optimum risk reduction of heart diseases, stroke and cancer in healthy individuals [5]. In Pakistan, there are numerous beverages and juice manufacturing companies which claim reasonable amount of Vitamin C in their products. The present study was designed to check levels of vitamin C in beverages/ juices and to verify manufacturers claim of added vitamin C.

2 Materials and Methods

2.1. Sampling locations

For the determination of ascorbic acid (AA) in juices and beverages, available brand of juices (packs, tin and bottles) were purchased from five locations in Islamabad. In addition powder sample used as soft drinks (Limopani and Tang) and Vitamin C tablets (CaC^{+}) were also obtained from the local market for AA measurements. Samples were categorized in to four groups according to the manufacturing companies and prices. Group A contains juice samples from international companies, Group B comprises of reputable Pakistani companies and low price juices of local companies were placed in Group C. Similarly, samples of carbonated drinks, powdered soft drinks and vitamin C tablets were placed in Group D. Table 1 gives details of groups and samples.

2.2. Sample Preservation and Processing

Immediately after collection, samples were stored at low temperature in a refrigerator. Pulpy samples were centrifuged at 3000 RPM (revolution per minute) in a centrifuge model WIROWKA. The supernatant was collected in another beaker and was filtered through Whatmann filter paper No.44. Powdered samples were dissolved in double distilled water, while the rest of the clear samples were neither centrifuged nor filtered. The tablets of vitamin C were dissolved in 100 ml of doubles distilled water.

Table 1. Sample categorization according to manufacturing company.

Group	Sample Code	Brand	Flavour
A	C1	Nestle	Orange
	C5	Olfruite	Apple
	C 7	Rani float	Mango
	C13	Minute Maid	Orange
	C14	Minute Maid	Orange
	C17	Flavors of Sammi	Strawberry
	C20	Nestle	Grapes
B	C6	Fresher	Falsa(cranberry)
	C8	Twist	Mango, banana
	C9	Fruiticana	Orange
	C10	Happy Time	Mango pulp
	C11	Slice	Mango
	C15	Sipley	Mango
	C18	Friss	Guava
C	C2	Shezan	Mix fruit
	C3	Country	Peach
	C4	Tops	Mango
	C12	Pine	Mango
	C16	Shezan	Apple
	C19	Sunday	Apple
Group D	C21	Marinda	Orange
	C22	7up	Lemon
	C23	Tang	Orange
	C24	Limopani	Lemon
	C25	CaC Tablet	Orange

2.3. Measurement of Physical Parameter

pH, Electrical Conductivity (EC) and Total Dissolved Solids (TDS) of samples were measured with portable meters. pH was measured with a pH meter (model Crison pH 25), TDS and EC were measured with the help of WTW conductivity meter (model LF 95). Each measurement was made in triplicate and the meters were calibrated with standard solution after every five measurements [6].

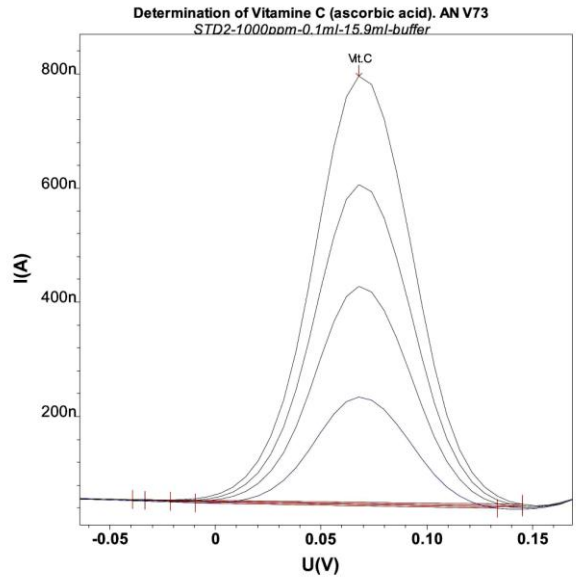

Figure 1. Plot of different Ascorbic Acid standards.

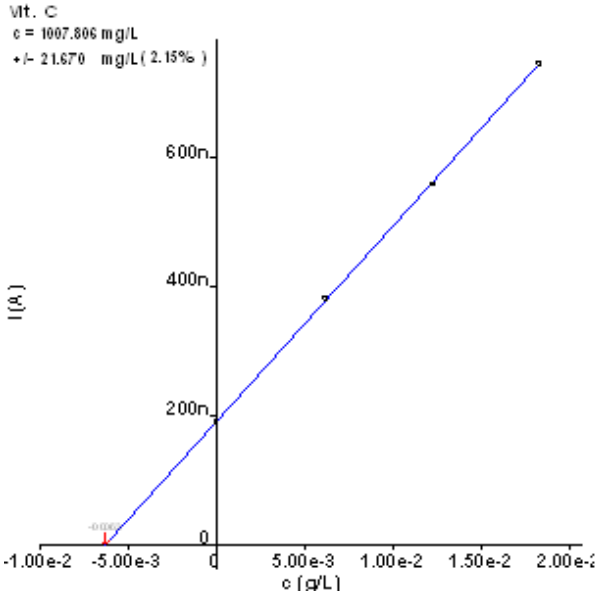

Figure 2. Calibration Curve of Ascorbic Acid.

2.4. Experimental

Polarograph 797 Computrace (Mehtrohm) was used for the determination of AA in different samples. It consists of three electrode assembly; a Dropping Mercury Electrode (DME) as working electrode, a reference electrode and platinum as a counter electrode. DC polarograms were recorded by the system software. All reagents used were of analytical grade. The mercury used in the dropping mercury electrode was obtained from BDH. Standard stock solutions of ascorbic acid were prepared with triply distilled water. A Platinum disc electrode (2 mm diameter) was used as working electrode. The reference electrode was a saturated calomel electrode (SCE). The potentiostate enables control of the potential of the working electrode, with respect to the reference electrode as well as measurement of the current that flows between the working electrode and the counter electrode. Standard ascorbic acid solutions concentrations ranging between 100-1000 ppm were prepared from the stock solution.

AA was determined by measuring the peak current and plotting a calibration graph. In order to verify the accuracy of the developed method for AA determination, standard addition method was applied for an analyzed sample. The obtained analytical signal was corrected by taking into account the sample dilution originating from the three additions of standard AA solution. The calibration curves for AA standards are shown in Figures 1 and 2.

3. Results and Discussion

3.1 Physiochemical Analysis

The pH, EC and TDS were obtained for all juice samples and are shown in Table 2. In group A, pH of samples lie in the range of 2.7 to 4.1. Highest pH was recorded in sample C1 while lowest was observed in sample C17. In group B pH of samples lie in the range of 3.1 to 4.1. Highest pH was recorded in sample C10 while lowest in sample C11. In group C pH of samples lie in the range of 3.1 to 3.8. Highest pH was recorded in sample C19 and lowest was observed in sample C12. Similarly, in group D pH of samples lie in the range of 2.62 to 4.75. Highest pH was recorded in sample C25 while lowest pH was observed in sample C23.

Electrical Conductivity of group A samples lie in the range of 218 to 2451 µS/cm. Highest EC was recorded in sample C1 while lowest EC was observed in sample C17. In group B, range of EC was 415.5 to 1998 µS/cm. The highest EC was recorded in sample C6 while lowest EC was observed in sample C8. EC of group C samples lie in the range of 389.3 to 1520 µS/cm. Highest EC was recorded in sample C4 while lowest EC was observed in sample C16. Similarly, in group D the EC range was between 1.223 to 947 µS/cm. The highest EC was recorded in sample C24 while lowest EC was observed in sample C23.

Table 2. pH. Electrical Conductivity and Total Dissolved Solids measured in test samples.

Sample Group & Code	pH	EC (µS/cm)	TDS (mg/l)
Group A			
C1	4.1±0.1	2451±0.5	1862.7±0.5
C5	3.1±0.1	714.4±0.5	618.0±0.5
C7	3.4±0.1	675.2±0.5	609.0±0.5
C13	3.4±0.1	582.5±0.5	442.7±0.5
C14	2.8±0.1	682.3±0.5	518.5±0.5
C17	2.7±0.1	218.0±0.5	907.0±0.5
C20	3.8±0.1	690.0±0.5	580.0±0.5
Range	2.7–4.1	218-2451	442.7-1862.7
Group B			
C6	3.2±0.1	1998±0.5	1445±0.5
C8	3.7±0.1	415.5±0.5	634.0±0.5
C9	3.6±0.1	895.9±0.5	680.9±0.5
C10	4.1±0.1	1162±0.5	883.1±0.5
C11	3.1±0.1	736.6±0.5	559.8±0.5
C15	3.9±0.1	943.9±0.5	717.3±0.5
C18	3.8±0.1	709.1±0.5	538.9±0.5
Range	3.1-4.1	415.5-1998	538.9-1445
Group C			
C2	3.3±0.1	856.0±0.5	546±0.5
C3	3.4±0.1	1118±0.5	734±0.5
C4	3.5±0.1	1520±0.5	633±0.5
C12	3.1±0.1	736.6±0.5	559±0.5
C16	3.3±0.1	389.3±0.5	711±0.5
C19	3.8±0.1	690.0±0.5	580±0.5
Range	3.1-3.8	389.3-1520	546-734
Group D			
C21	2.71±0.1	489.2±0.5	602±0.5
C22	3.33±0.1	760±0.5	564±0.5
C23	2.62±0.1	1223±0.5	1182±0.5
C24	2.92±0.1	947±0.5	1027±0.5
C25	4.75±0.1	4772±0.5	4294±0.5
Range	2.62-4.75	760-1223	564-4294

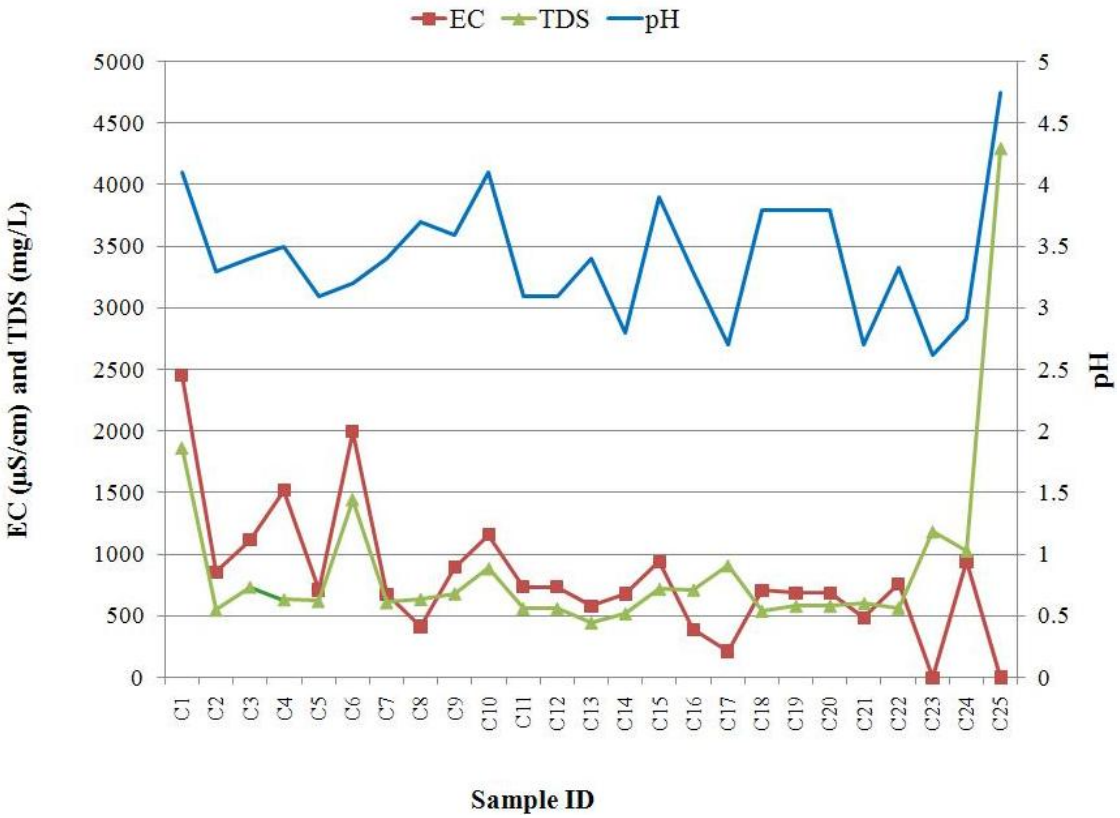

Figure 3. Plot of physical parameters.

Total Dissolved Solids of group A samples lie in the range of 442.7 to 1862.7 mg/l. The highest TDS was recorded for sample C1 while lowest TDS was observed in sample C13. In group B, TDS of samples lie in the range of 538.9 to 1445 mg/l. The highest TDS was recorded for sample C6 while lowest TDS was observed in sample C18. In group C, TDS of samples lie in the range of 546 to 734 mg/l**.** The highest TDS was recorded in sample C3 while lowest TDS was observed in sample C2. Similarly, in group D the TDS of samples lie in the range of 564 to 4294 mg/l**.** The highest TDS was recorded for sample C25 while lowest TDS was observed in sample C22.

Figure 3 shows a plot of EC, pH and TDS values for all of the samples studied. It appears from this figure that there was a significant variation of these parameters in orange and grape flavored juices, however, for lemon, apple and mango juices EC and TDS were quite uniform but pH of these samples varied to some extent. These variations may be attributed to differences in fruits used, the ingredients added by different companies or poor storage conditions which might lead to degradation of juice material.

3.2. Ascorbic Acid Content

In the following paragraphs the AA values obtained will be presented and discussed in details. All the values were converted to mg/pack from mg/L. Some manufacturers labeled vitamin C concentration in mg while some have stated percent values of daily recommended values while the remainder only mentioned “enriched with vitamin C” and did not mention its exact values.

Group A

Results of reported and measured values of group A are shown in Table 3. Measured values of AA in this category when compared with the reported values of AA were found to be quite inconsistent. Comparable values were observed for sample C1, C14 and C17, whereas determined values were significantly different from reported values for samples C5, C7 and C20. Sample C20 gave surprisingly different results, no AA was observed in C20 whereas manufacturer claims an amount of 500 mg /pack.

Table 3. AA concentration of Group A samples.

Sample code	AA Concentration (mg/pack)	
	Reported AA Concentration	Measured AA Concentration
C1	17 (85)	26.53 ± 0.47 (132.67)
C5	Enriched	1.15 ± 0.09 (5.75)
C7	Enriched	5.50 ± 0.13 (22.91)
C13	30.0 (75)	130.9 ± 8.43 (327.38)
C14	22.0 (91.6)	19.11 ± 1.57 (79.63)
C17	42 (168)	45.95 ± 0.95 (183.79)
C20	500 (1000)	BDL

BDL= Below Detection Limit (<3ppm)

Values in parenthesis shows mg/L

Group B

Table 4 shows the AA concentration in group B juices. These juices are of reputed Pakistani companies and in majority of juice packs manufacturers have mentioned "Enriched with vitamin C" but no exact amount is mentioned. However, measured values show that the concentration of AA in samples are either negligible or below detected limit. In only one sample (C18) out of seven, the measured value of vitamin C is comparable with what is written on the pack. In some cases no value of vitamin C is reported while in others it is assumed that "enriched with Vitamin C" implies that measureable amount of vitamin C is added). In this sample C18 (Guava juice) the measured value shows high concentration of AA which may be due to the fact that Guava is a good source of vitamin C [2].

Table 4. AA concentration of Group B samples.

Sample code	AA Concentration (mg/pack)	
	Reported AA Concentration	Measured AA Concentration
C6	Enriched	BDL
C8	Enriched	0.57 ± 0.19 (2.26)
C9	Enriched	BDL
C10	Enriched	25.46 ± 3.05 (79.56)
C11	Enriched	BDL
C15	31.0 (124)	89.35±5.35 (357.39)
C18	Enriched	668.56 ± 0.19 (1337.11)

BDL= Below Detection Limit (<3ppm)

Values in parenthesis shows mg/L

Group C

Table 5 shows the concentration of AA in juices of local Pakistani companies. Manufacturer's claim of vitamin C in the juice packs are very high while the determined values are negligible or below detected limits. Concentration of AA in four out of six juice samples is below detected limit while two samples have negligible amounts as compared to their reported values.

Table 5. AA concentration of Group C samples.

Sample code	AA Concentration (mg/pack)	
	Reported AA Concentration	Measured AA Concentration
C2	24 (96)	3.52 ±0.56 (14.08)
C3	100 (400)	1.29 ±0.19 (5.15)
C4	30.6 (153.0)	BDL
C12	Enriched	BDL
C16	24 (170.4)	BDL
C19	50 (200)	BDL

BDL= Below Detection Limit (<3ppm)

Values in parenthesis shows mg/L

Group D

AA concentration in two samples of carbonated drinks, powdered soft drinks and Vitamin C tablets is given in Table 6. In one carbonated soft drink of orange flavor, manufacturer has mentioned ascorbic acid in ingredients but no vitamin C was detected. Vitamin C tablets that are commonly used as a source of vitamin C were also tested and a significant value of AA was determined. Comparable concentration of AA were determined in two powdered soft drinks that are commonly used as drinks in the summer season and are a good source of vitamin C.

Table 6. AA concentration of Group D samples.

Sample code	AA Concentration (mg/pack)	
	Reported AA Concentration	Measured AA Concentration
C21	Enriched	BDL
C22	-	BDL
C23	33 (165)	78.0±3.47 (390.44)
C24	75 (375)	57.96 ±0.33 (579.6)
C25	500*	561.3 ±17.08

BDL= Below Detection Limit (<3ppm)

Values in parenthesis shows mg/L

* Vitamin C Tablet

Table 7. Pearson correlation coefficients for all parameters studied.

All Data				
	pH	EC	TDS	Ascorbic Acid Concentration
pH	1.00	0.60	0.53	0.29
EC		1.00	0.95	0.39
TDS			1.00	0.43
Ascorbic Acid Concentration				1.00
Fruit				
	pH	EC	TDS	Ascorbic Acid Concentration
pH	1.00	0.55	0.48	0.22
EC		1.00	0.98	0.84
TDS			1.00	1.00
Ascorbic Acid Concentration				1.00

Correlation coefficients for the samples studied were obtained to see any relationship between the various physicochemical properties and the AA content. The samples were also grouped according to fruit type to see the effect if any of these variations. The data obtained is presented in Table 7.

A significant positive correlation has been observed between pH and EC (0.55) and pH and TDS (0.48) for fruit based analysis. Similarly fruit based analysis showed a positive correlation between EC and AA content (0.84) and TDS and AA concentration (0.40).

4. Discussion

The study reveals that juices of international brands (group A) do not have comparable amount of vitamin C, whereas in group B of reputed companies of Pakistan origin, most of the juices either have no vitamin C or their amounts were below detection limit. However, measured values of vitamin C in group D products are quite comparable with written values on pack. A plot of reported values versus measured values of the samples in which AA was detected and reported by manufacturer is shown in Figure 4.

No significant correlation is observed (R^2 =0.2) between reported and measured values. Absence or lower amount of vitamin C in juice packs may be due to two main factors. Firstly, either the manufacturer did not add vitamin C against their claim or vitamin C might have degraded. According to the literature data, the amount of vitamin C in different juices decreases during storage, depending on storage conditions, such as temperature, oxygen and light exposure [7,8].

AA addition is common in the manufacture of beverages, especially those made from fruit juices. AA not only restores nutritional value lost during processing, but also contributes to the products appearance and palatability. In the manufacture of fruit juices or purees from fruits such as apples and peaches, AA may be added during the crushing, straining, or pressing processes to prevent enzymatic browning of the raw fruits.

Nutritional quality of food during storage has become increasingly important. The loss of AA (vitamin C) might be a critical factor for the shelf life of some products as citrus juice concentrates, since vitamin C content of citrus juices undergoes destruction during storage [9-11]. AA is an important component of our nutrition and is used as an additive in many foods because of its antioxidant capacity. Thus, it increases quality and technological properties of food as well as nutritional value [12]. Degradation of AA proceeds via both aerobic and anaerobic pathways [9,13] and depends upon many factors such as oxygen, heat, light [14] and storage temperature and storage time [15].

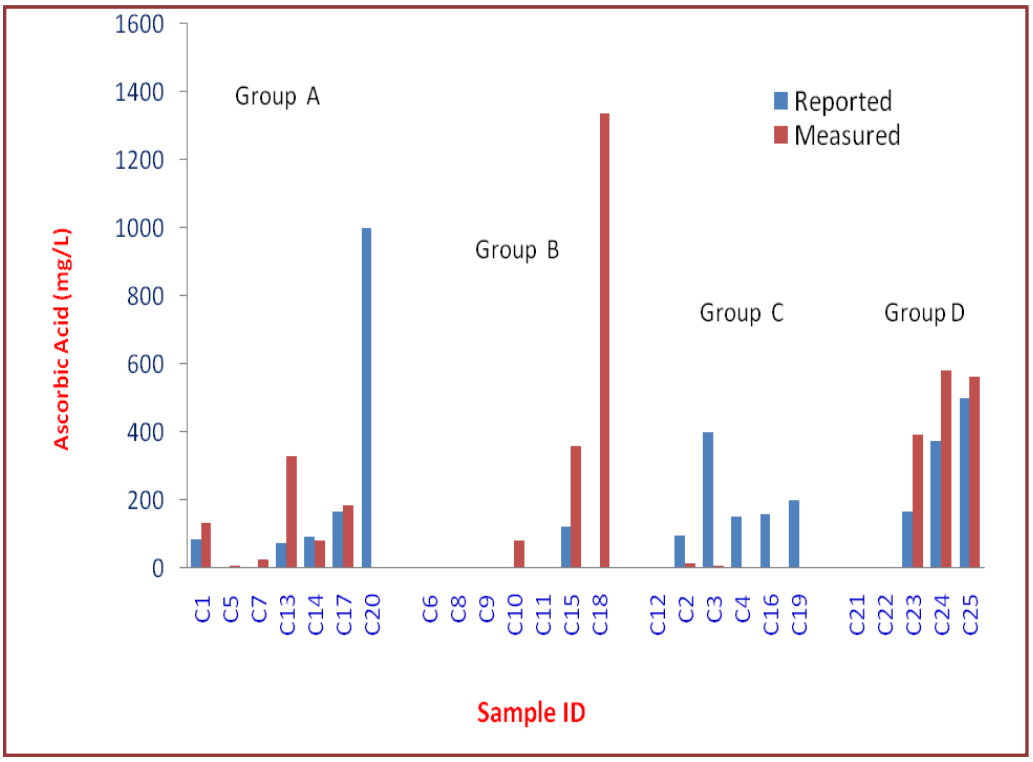

Figure 4. Plot of reported values of Ascorbic acid versus measured values.

In this study all the collected samples were well within expiring dates. As the samples were collected in June, it is suspected that due to discontinuous supply of electricity the storage conditions may not be good enough for proper preservation and resulted in degradation of AA. Oxidation of AA occurs mainly during the processing of citrus juices [13], whereas, anaerobic degradation of AA mainly appears during storage [9,11] which is especially observed in thermally preserved citrus juices. It was reported that several decomposition reactive products occur via the degradation of vitamin C [16] and these compounds may combine with amino acids, thus result in the formation of brown pigments [12]. Hydrooxymethylfurfural (HMF) is one of the decomposition products of AA [11, 16]. It is used to evaluate severity of heat applied to fruit juices during processing and is taken into account for quality control [17]. Other pathways of HMF accumulation are known as degradation of reducing sugars [18] and Maillard reaction [19]. Since AA degradation causes browning which is the other problem of quality loss in citrus juices during storage [20] it is necessary to describe ascorbic acid degradation and investigate kinetics of AA loss in stored citrus juices.

5. Conclusions

On the basis of this study it is concluded that:

- In some brands of international companies the measured vitamin C was comparable with the reported values, while in other these values showed variation. Most Pakistani companies of good repute have no vitamin C in their products or their amounts were negligible against the manufacturer claim. Vitamin C was below detectable limits in juice packs of majority of local companies. It can be concluded that that either no vitamin C is added in juices or it may degrade during storage.
- Vitamin C concentration in vitamin C tablets and solid soft drinks is quite comparable with written values on pack expect for carbonated drinks.
- On the basis of this study it is recommended that strict QC be implemented at the factories to prepare goods which have properties as given on their labels.

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