‘‘ANALYSIS AND COMPARATIVE STUDY OF TOXIC AND ESSENTIAL HEAVY METALS, CITRIC AND ASCORBIC ACID ESTIMATION AND PHYSICOCHEMICAL PARAMETERS IN FRESH AND PACKAGED FRUIT JUICES COLLECTED FROM AMER AREA, JAIPUR (RAJASTHAN), INDIA’’

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Abstract: Fruit juices are widely consumed in many parts of India and are considered to be nutritive to the body. In this study, an analysis of four heavy metals: Fe, Zn, Pb, and Cd was done by FAAS method in fresh and packaged fruit juices of pineapple, orange, and pomegranate. The highest concentration of Pb was found to be 17.10 mg/L in the packaged pomegranate juice while a value of 21.46 mg/L Cd was observed in the fresh pineapple juice. All samples had Pb and Cd concentrations that exceeded the limit in drinking water imposed by World Health Organization (WHO) and United State Environmental Protection Agency (US-EPA), with a single exception. These concentrations of Pb and Cd are alarming and present potential health hazard. There were significant concentrations of Fe and Zn. The samples were also tested for reducing sugar and phosphate and were positive. The pH was found to be an important physico-chemical parameter. They also contained ascorbic acid with highest value in the fresh pure orange juice sample and citric acid with highest value in the fresh orange juice sample. The study therefore indicated the need for improvement of quality control in the fruit juices consumed in the area.

KEYWORDS: Analysis, physico-chemical, heavy metal, citric acid, ascorbic acid, fruit juice

1. INTRODUCTION

As part of human nutrition sources, fruits are consumed by all people in the worldwide. Besides their taste which make people love them, fruits contain various active chemical constituents capable of promoting human health. Traditional drinks including fresh fruit juices are thirst quenching and nutritionally enriched food category of Asiatic region extracted at mobile and stationary carts and offered for sale in loose form. In most countries, the hot climate means that the intake of liquids must be high to compensate for the expected losses from respiration [1-4]. It is widely accepted that a diet rich in fruits that are good sources of vitamin C, carotenoids, minerals (especially Mg, K) and various kinds of antioxidants and dietary fibre (pectin) is protective against degenerative and chronic diseases such as cancer and cardiovascular diseases. On the other hand, the quality of fruit products is diminished with increasing concentration of toxic compounds, environmental pollutants (especially pesticides), polychlorinated biphenols (PCB’s) and heavy metals, especially Pb and Cd [5-9]. Heavy metals composition of food is of interest because of their essential or toxic nature. For example, iron, zinc, copper, chromium, cobalt, and manganese are essential, while lead, cadmium, nickel, and mercury are toxic at certain levels [10-11]. Heavy metals are those with atomic weights from 63.546 to 200.59, and specific weight higher than 4. Heavy metals have atomic weight and density almost five times that of water. A toxic heavy metal is any relatively dense metal or metalloid that is noted for its potential toxicity, especially in environmental contexts. In other words; heavy metal is a term, given to the group of metals and metalloids with atomic density greater than $5g/cm^3$, usually associated with pollution and toxicological problems. The term has particular application to Cadmium, Mercury, Lead and Arsenic, all of which appear in the World Health Organization’s list of 10 chemicals of major public concern [12-14]. A lot of analysis has been done on heavy metals in different substances in many places, but the high demand for fruit juice in India require frequent validation for safety issues. Some of these works include: N. Eka et al [1] on the validation and quantitative analysis of cadmium and lead in snake fruit by flame atomic absorption spectrophotometry**,** Saeed Akhtar et al [2] have done work on safety assessment of street vended juices in Multan-Pakistan: A study on prevalence levels of trace elements**.** The safety study of juices depicted higher concentration of lead (Pb) and cadmium (Cd) breaching international safety limits implemented in the country. Among other works include: Anwar et al [3] ''comprehensive study for determination of heavy metals including trace (Cr, Fe, Zn, Ni, Mn, Co, Cu) and toxic (Pb, Cd) metals in variety of fruit juices and also studied the impact of these on human health'', Ithar Kamil Al-Mayaly [4] on the determination of some heavy metals in some artificial fruit juices in Iraqi local markets, Z. Krejpcio et al [5] on the safety of fresh fruits and juices available on the Polish Market as determined by heavy metal residues, and A. I. Ajai et al [15] on the work ''determination of trace metals and essential minerals in selected fruit juices in Minna, Nigeria''. The main aim of this research work is to determine the concentrations of toxic and essential heavy metals in some juice samples collected from Amer area, Jaipur, India, in order to assess the quality or safety of the juices consumed in the area. This will be achieved through the following objectives: **1.** To collect fresh and packaged Juice samples of three fruits (Pineapple, Orange and Pomegranate) from Amer area, Jaipur. **2.** To analyze two toxic heavy metals; Lead (Pb) and Cadmium (Cd) in the juice samples by AAS method.**3.** To analyze two essential heavy metals; Iron (Fe) and Zinc (Zn) in the juice samples by AAS method. **4.** To compare the results obtained with standard guidelines. **5.** To determine some physicochemical parameters. **6.** To determine the presence of reducing sugar and phosphate. **7.** To estimate citric and ascorbic acid contents.

2. MATERIALS AND METHODS

To ensure reliability of the results, samples were carefully handled to avoid contamination. All glass wares used were washed with chromic acid, tap water, distilled water and finally dried in an oven overnight.

2.1. Sample Collection.

Six (6) fruit juice samples consisting of fresh and packaged juices of three (3) fruits (pineapple, orange and pomegranate) were purchased from Amer area, Jaipur. They were labeled as follows:

Sample 1: packaged pineapple fruit juice
Sample 2: packaged orange fruit juice
Sample 3: packaged pomegranate fruit juice
Sample 4: fresh pineapple fruit juice
Sample 5: fresh orange fruit juice
Sample 6: fresh pomegranate fruit juice

2.2. Physicochemical Analysis

2.2.1. Conductivity measurement

The conductivity of the juice samples (undigested) was measured with an auto digital conductivity meter model LT–16 at Chemistry Labotatory, NIMS Institute of Engineering and Technology, Jaipur. The instrument with a cell constant 0.970 was turned on and allowed to warm up for at least 15 – 30mins. The electrode of the instrument was rinsed with deionized water and then with the liquid juice sample. The conductivity of the samples was then taken at 30ºC and recorded. The same procedure was applied for each sample.

2.2.2. pH measurement

This was done by the dipstick method. A pH paper was dipped into 2 ml of the juice sample contained in a test tube. The change in colour of the pH paper was noticed and compared with the standard pH scale of the kit [18].

2.2.3. Density measurement

The density of the juice samples was measured using R.D bottle. The R.D bottle is slightly round bottomed type of glass vessel. It is fitted with a glass or plastic cork containing a fine capillary. The R.D. bottle was first washed with chromic acid solution and then with distilled water and finally with alcohol. It is then dried and weighed. The R.D. bottle is then filled with distilled water and stoppered. There was no air bubble inside the R.D. bottle. The R.D. bottle is then again weighed. Water is then poured out and washed with alcohol and dried. The R.D. bottle is then filled with experimental liquid as before and weighed again.

Let: Mass of empty R.D. bottle $= w_1$ gm
Mass of R.D. bottle + water $= w_2$ gm
Mass of R.D. bottle + liquid $= w_3$ gm

Then,
$$\frac{\text{density of liquid } (d_1)}{\text{density of water } (d_2)} = \frac{w_3 - w_1}{w_2 - w_1}$$

$$d_1 = \frac{w_3 - w_1}{w_2 - w_1} \times d_2$$

2.3. Qualitative Analysis

2.3.1. Test for reducing sugar by Benedict's test

Benedict's solution was used to test for the presence of reducing sugar. In this procedure, 3 ml of the sample is taken into a test tube and 2 ml of Benedict reagent is added. The test tube is heated in a water bath for 5 min and the formation of reddish colour confirmed the presence of sugar in the juice sample [18]. Depending on the amount of sugar present, Benedict test may give colour that progress to green, yellow, orange, red and then a dark red or brown.

2.3.2. Test for reducing sugar by Fehling's test

The presence of reducing sugar was tested for using Fehling solution. In this test, 3 ml of the sample is taken in a test tube and 2 ml of a mixture of Fehling's A and Fehling's B solutions in equal amount is added. The test tube is heated in a water bath for 10 min and the appearance of brown, rust or red precipitation confirmed the presence of reducing sugar [18]. If reducing sugars are not present, the solution will remain blue or green.

2.3.3. Test for phosphates

3 ml of sample for each juice was taken into separate test tubes. 2ml of ammonium molybdate followed by 2 ml of concentrated nitric acid (HNO_3) were added. The solution is then heated in a water bath for 10 min and appearance of canary-yellow precipitate confirmed the presence of phosphate ions in juice samples [18].

2.4. FAAS Metal Analysis

2.4.1. Sample Digestion prior to AAS Analysis

Prior to quantitation of analyte by Atomic Absorption Spectrometry, it is usually necessary to destroy the organic matrix and bring the element into clear solution. For this reason the juice samples were first digested with chemicals where the organic matrix of juice is destroyed and leave the element into a clear solution [16]**.** The method employed by Ajai et al [15] was used for sample digestion with a slight modification; 10mL of concentration nitric acid (HNO_3) is added to 5mL of the fruit juice sample in a closed vessel to avoid loss by volatilization of the metals, especially Pb and the solution is heated on a hot plate in a fume hood for 1 hour. This is then allowed to cool and filtered into a 50mL volumetric flask and make up to 50mL mark with deionized water and stored in polyethylene bottles prewashed with de-ionized water prior to analysis. Red fumes observed during digestion are indicating the release of nitric acid [17]

2.4.2 Analysis of Samples

The digested fruit juice samples were analyzed in terms of two toxic heavy metals (Pb and Cd) and two essential heavy metals (Fe and Zn) using Flame Atomic Absorption Spectrophotometer on air – acetylene flame at wavelength 283.2 nm, 228.8 nm, 248.3nm and 213.86nm respectively.

2.5. Titrimetric Analysis

2.5.1. Determination of Ascorbic Acid

The method used to determine the concentration of vitamin C or ascorbic acid in the juice samples is by redox titration using 0.005M iodine solution and 1% starch indicator with 20mL juice sample. For each juice sample, 20mL each of pure and diluted juice were analyzed separately. The juice samples were diluted by 1+9 dilution in water.

2.5.2. Determination of Citric Acid

The total acidity of a fruit juice is ordinarily determined by titrating a known volume of juice with a standard solution of NaOH, with phenolphthalein as indicator, the result being expressed as citric acid. The NaOH used is 0.1M and the juice samples were diluted by 1+9 dilution in water. 20mL each of the diluted juice samples were used with six drops of the indicator.

3. RESULTS AND DISCUSSIONS

3.1. Physicochemical Analysis

Tab**le 1:** Results of Conductivity Measurement for the Juice Samples at 30^oC

Juice Sample	Conductance in mmhos/cm
Sample 1	1.622±0.025
Sample 2	1.888±0.029
Sample 3	1.838±0.038
Sample 4	1.230±0.014
Sample 5	2.343±0.025
Sample 6	2.560±0.053

Values presented as mean ± standard deviation

Table 1 reveals the results of conductance for the juice samples with sample 6 having the highest value while sample 4 being the lowest. The order of the samples with respect to conductance is: sample 6 > sample 5 > sample 2 > sample 3 > sample 1 > sample 4. Conductivity measurements are used routinely in many industrial and environmental applications as a fast, inexpensive and reliable way of measuring the ionic content in a solution. The SI unit of conductivity is siemens per meter (S/m) and, unless otherwise qualified, it refers to 25 °C. Often encountered in industry is the traditional unit of μS/cm. 10^6 μS/cm = 10^3 mS/cm = 1 S/cm. The values in μS/cm are lower than those in μS/m by a factor of 100 (i.e., 1 μS/cm = 100 μS/m). Sometimes encountered is mho (reciprocal of ohm): 1 mho/m = 1 S/m.

Table 2: Results of pH determination for the Juice Samples using pH paper test strips.

Juice Sample	pH value	Strength
Sample 1	5.67	Weakly acidic
Sample 2	5.33	Weakly acidic
Sample 3	3.00	Strongly acidic
Sample 4	5.67	Weakly acidic
Sample 5	5.00	Weakly acidic
Sample 6	2.33	Strongly acidic

Values presented as mean of triplicate determinations

Table 2 reveals the results of pH test for the juice samples which follow the order: sample 1 = sample 4 > sample 2 > sample 5 > sample 3 > sample 6. All samples have pH values below 7 and thus acidic. Samples 3 and 6 both of which contain pomegranate juices are the most acidic samples (Strongly acidic) while the other four samples are weakly acidic. The critical factors affecting the spoilage of juices include juice pH, oxidation reduction potential, water activity, availability of nutrients, presence of antimicrobial compounds, and competing microflora. Among these factors, pH and water activity are the most influential factors affecting the spoilage of juices [19]. Fruit juices have pH in the acidic range (<4.5) serving as important barrier for microbial growth. However, food borne pathogens such as *E. coli* and *Salmonella* survive in acidic environment of fruit juices due to acid stress response. Therefore, in the last two decades a number of food borne outbreaks associated with unpasteurized fruit juices have been documented in many countries. The source of entry of microorganisms into fresh fruit juices is from environment exposure and soil [19]. Many microorganisms such as acid tolerant bacteria and fungi (moulds, yeasts) use the Microflora normally present on the surface of fruits during harvest and postharvest processing as a substrate for their growth [19]. Also, according to the FDA; the acid-tolerant pathogens are not the only harmful microorganism that could occur in juice as there are other pathogens that may occur in low-acid juices (pH greater than 4.6). Thus, as the pH of 4 of the 6 samples is at least 5, a serious potential hazard exists.

Table 3: Results of density measurement for the Juice Samples.

Juice Sample	Density (g/mL)
Sample 1	1.055
Sample 2	1.048
Sample 3	1.056
Sample 4	1.055
Sample 5	1.052
Sample 6	1.057

Values presented as mean of triplicate determinations

Table 3 reveals the results of density measurement for the juice samples with a high level of precision in the values of sample 1, sample 3, sample 4, sample 5 and sample 6 while sample 2 being less precise than the rest of the samples. Density may be relevant to buoyancy, purity and packaging.

3.2. Qualitative Analysis

Table 4: Results of Qualitative Analysis for the Juice Samples

Juice Sample	Reducing Sugar by Benedict's test	Reducing Sugar by Fehling's test	Phosphate
Sample 1	Positive	Positive	Positive
Sample 2	Positive	Positive	Positive
Sample 3	Positive	Positive	Positive
Sample 4	Positive	Positive	Positive
Sample 5	Positive	Positive	Positive
Sample 6	Positive	Positive	Positive

Table 4 reveals the results of qualitative analysis of reducing sugar and phosphate in the juice samples. The reducing sugar test is done by Benedict's and Fehling's tests. Both tests are used to distinguish between reducing and non-reducing sugars in that; reducing sugars give positive Benedict's or Fehling's test. This is achieved by the observation of color change after adding juice samples and reagents (Benedict's or Fehling's solutions) and then heating. As seen from the table, all juice samples show positive sugar tests. The table also indicated that all the juice samples were positive for phosphate as seen by the appearance of canary – yellow precipitate after heating. Phosphorous is an important element for the body. It forms a major constituent of the DNA, cell membrane layer and channels and is also vital for teeth and bone formation. Phosphorous naturally exist as phosphates which are acidic in nature and can be obtained from dietary sources [19]. A **phosphate** (PO^{3-}_4) is an inorganic chemical and a salt-forming anion of phosphoric acid. In organic chemistry, a phosphate, or organophosphate, is an ester of phosphoric acid. Phosphate was present in all the juice samples and thus could be beneficial especially in children for the development of teeth and bones.

Sugars are classified as reducing and non-reducing based on their ability to act as a reducing agent. A reducing agent donates electrons during a redox reaction and is itself oxidized. The aldehyde functional group is the reducing agent in reducing sugars. Reducing sugars are sugars that have the hemiacetal or hemiketal functional group somewhere in their molecular structure. Either of these two functional groups will be in equilibrium with a free aldehyde group which will be very easily oxidized to a carboxylic acid. This oxidation will be accompanied by the reduction of the oxidizing agent often copper(II) ion or silver(I) ion, the copper(II) ion is reduced to copper(I) ion and the silver ion to silver metal.

Generally, Benedict's test detects the presence of aldehydes and alpha-hydroxy-ketones, also by hemiacetal, including those that occur in certain ketoses. Thus, although the ketose fructose is not strictly a reducing sugar, it is an alpha-hydroxy-ketone, and gives a positive test because it is converted to the aldoses glucose and mannose by the base in the reagent. The color of the obtained precipitate gives an idea about the quantity of sugar present in the solution, hence the test is semi-quantitative. A greenish precipitate indicates about 0.5 g% concentration; yellow precipitate indicates 1 g% concentration; orange indicates 1.5 g% and red indicates 2 g% or higher concentration. A general diagram is shown below.

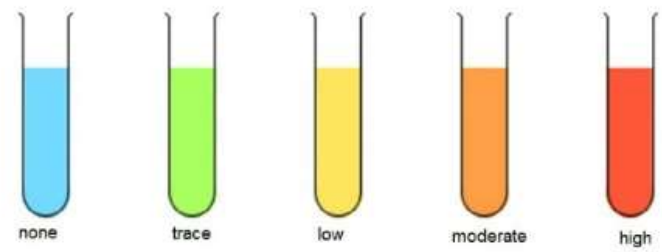

Figure 1: The semi-quantitative colors of sugar with Benedict's reagent.

Similarly, in the Fehling's test the presence of aldehydes but not ketones is detected by reduction of the deep blue solution of copper (II) to a red precipitate of insoluble copper oxide. The test is commonly used for reducing sugars but is known to be NOT specific for aldehydes. For example, fructose gives a positive test with Fehling's solution as does acetoin (3-hydroxybutanone) $CH_3CH(OH)COCH_3$. A general sample image for the three common tests for aldehydes is shown below:

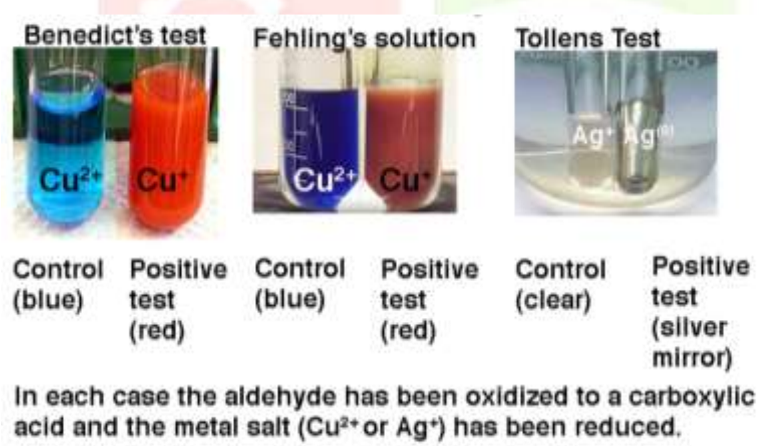

Figure 2: The three common tests for aldehydes.

Reducing carbohydrate + Fehling's or benedict's solution (**Cu^{2+}**)
(Blue or green)

$\longrightarrow$ Oxidized carbohydrate + Cu^{+}
(Red, brown or orange)

Example:

$C_6H_{12}O_6$ (Glucose) + $2Cu(OH)_2 \longrightarrow C_6H_{12}O_7$ (Gluconic acid) + Cu_2O + H_2O
(Red)

3.3. FAAS Metal Analysis

Table 5: Results of AAS Essential Heavy Metal Analysis (Iron and Zinc) for the Juice Samples at 324.75nm and 213.86nm respectively.

S.No.	Sample I.D.	Mean Concentration of Metal in mg/L Fe	Zn
1	Sample 1	17.94	17.52
2	Sample 2	52.03	17.38
3	Sample 3	10.63	14.63
4	Sample 4	12.97	14.79
5	Sample 5	9.966	15.01
6	Sample 6	16.91	15.40

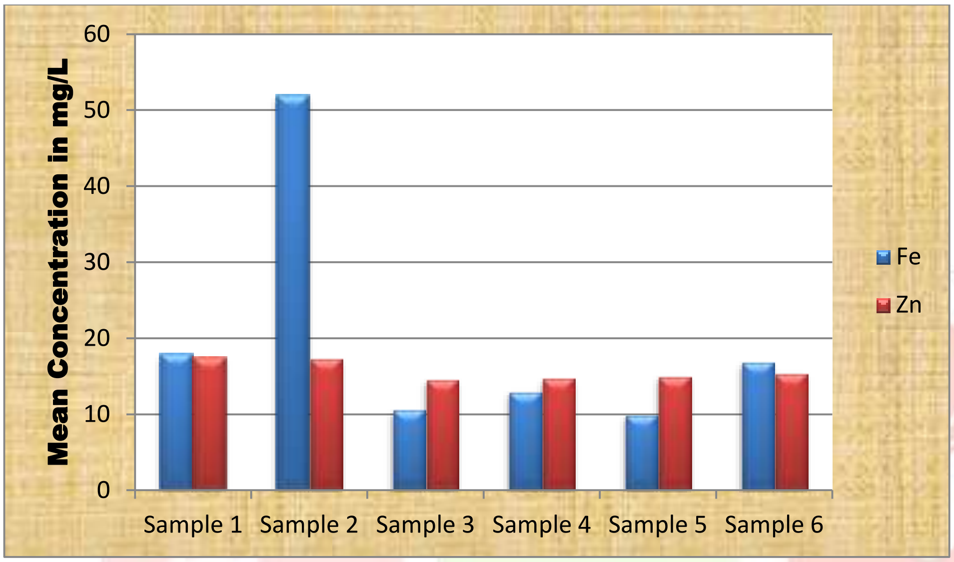

Figure 3: The mean concentration of iron (Fe) and Zinc (Zn) for the juice samples in mg/L.

Table 5 shows the results of essential heavy metal analysis of Fe and Zn by AAS for the Juice Samples at 324.75nm and 213.86nm respectively. The mean concentration of Fe follow the order: sample 2 > sample 1 > sample 6 > sample 4 > sample 3 > sample 5 while that of Zn follow the order: sample 1 > sample 2 > sample 6 > sample 5 > sample 4 > sample 3. Thus samples 2 and 1 have the highest concentration of Fe and Zn respectively. The chart in Figure 3 represents these concentrations for both Fe and Zn in all six samples. In the context of nutrition, a mineral is a chemical element required as an essential nutrient by organisms to perform functions necessary for life. Minerals originate in the earth and cannot be made by living organisms. Plants get minerals from soil. Most of the minerals in a human diet come from eating plants and animals or from drinking water. As a group, minerals are one of the four groups of essential nutrients, the others of which are vitamins, essential fatty acids, and essential amino acids. The five major minerals in the human body are calcium, phosphorus, potassium, sodium, and magnesium. All of the remaining elements in a human body are called "trace elements". The trace elements that have a specific biochemical function in the human body are sulfur, iron, chlorine, cobalt, copper, zinc, manganese, molybdenum, iodine and selenium. Hence, this study indicated significant amount of Fe and Zn in the juice samples which are essential to the body.

Table 6: Results of AAS Toxic Heavy Metal Analysis (Cadmium and Lead) for the Juice Samples at 228.8 nm and 283.2 nm respectively.

S.No.	Sample I.D.	Mean Concentration of Metal in mg/L Cd	Pb
1	Sample 1	5.563	3.789
2	Sample 2	0.095	0.069
3	Sample 3	0.244	17.10
4	Sample 4	21.46	1.512
5	Sample 5	5.929	1.990
6	Sample 6	5.818	4.683

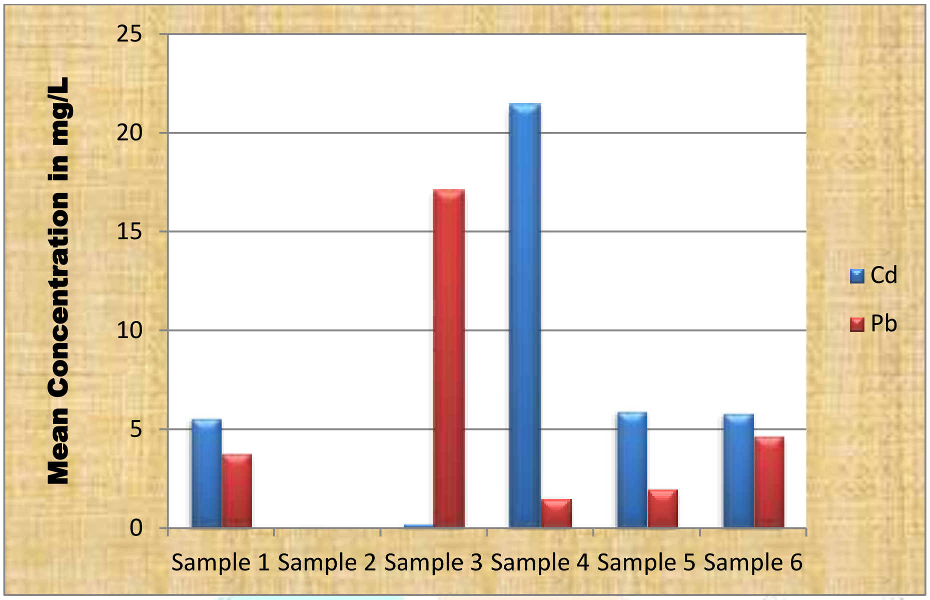

Figure 4: The mean concentration of cadmium (Cd) and lead (Pb) for the juice samples in mg/L.

Table 6 shows the results of toxic heavy metal analysis of Cd and Pb by AAS for the Juice Samples at 228.8 nm and 283.2 nm respectively. The mean concentration of Cd follow the order: sample 4 > sample 5 > sample 6 > sample 1 > sample 3 > sample 2 while that of Pb follow the order: sample 3 > sample 6 > sample 1 > sample 5 > sample 4 > sample 2. Thus sample 2 has the lowest concentration of both Cd and Pb, while samples 3 and 4 have alarming concentrations of Pb and Cd respectively. The chart in Figure 4 represents these concentrations for both Cd and Pb in all six samples. Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. The criteria used, and whether metalloids are included, vary depending on the author and context. In metallurgy, for example, a heavy metal may be defined on the basis of density, whereas in physics the distinguishing criterion might be atomic number, while a chemist would likely be more concerned with chemical behaviour. More specific definitions have been published, but none of these have been widely accepted. A density of more than 5 g/cm^3 is sometimes quoted as a commonly used criterion. Some heavy metals are either essential nutrients (typically iron, cobalt, and zinc), or relatively harmless (such as ruthenium, silver, and indium), but can be toxic in larger amounts or certain forms. Other heavy metals, such as cadmium, mercury, and lead, are highly poisonous. Potential sources of heavy metal poisoning include mining, tailings, industrial wastes, agricultural runoff, occupational exposure, paints and treated timber. Hence, this study indicated significant amount of Cd and Pb in the juice samples (with exception of sample 2) which are toxic or harmful to the body.

Table 7: Drinking water contaminants and maximum admissible limits by different organization.

Heavy metal Contaminates	US-EPA Limits (mg/L)	WHO Limits (mg/L)
Cr	0.1	0.05
Fe	–	–
Zn	5	NGL
Ni	0.1	0.07
Mn	0.05	0.4
Co	0.1	–
Cu	1.3	2
Pb	1.5	0.01
Cd	0.005	0.003

NGL: no guide line, because it occur in drinking water at concentrations well below those at which toxic effects may occur. WHO, 2008; US-EPA, 2008; Dehelean and Magdas, 2013[3].

By comparing the values in table 5, 6 and those in table 7 for drinking water by USEPA and WHO as standard guidelines; it is observed that the analyzed samples in this study had concentrations that exceeded those in the reference standards for Pb and Cd, except that one of the six samples analyzed had less than 1.5mg/L for Pb as set by USEPA. Though no guideline was set for Fe by both organizations, the concentrations observed in the samples were high while all samples had concentrations of Zn that exceeded the limit set by USEPA.

3.4. Titrimetric Analysis

Table 8: Results of Ascorbic Acid Determination

Sample no.	mg ascorbic acid/20mL[a]	mg ascorbic acid/20mL[b]
Sample 1	0.44	0.62
Sample 2	1.06	1.23
Sample 3	0.35	1.06
Sample 4	0.71	2.20
Sample 5	0.97	4.84
Sample 6	0.79	2.11

a= 20mL of diluted juice (1+9 dilution in water)
b= 20mL of pure juice (undiluted or concentrated)

In table 8, samples 1 – 3 represent packaged pineapple, orange and pomegranate while samples 4 – 6 represent fresh pineapple, orange and pomegranate. The table shows that both the fresh and packaged orange juices contained more vitamin c or ascorbic acid than pineapple and pomegranate. Thus citrus fruits are rich in vitamin c. Also, from the results obtained for ascorbic acid in *a* and *b* for all samples in the table, it can be clearly seen that the values for the pure juices are greater than those for the diluted samples. This is in accordance with the collision theory. The more the solution of a reactant is diluted, the slower the reaction will occur. The key thing to remember is that chemical reactions depend on reactant particles bumping into each other having sufficient energy (collision theory). The more concentrated a reactant is, the more likely it will bump into other reactants and produce chemical change. So it is expected that the diluted juice should contain less amount of the ascorbic acid than the pure juice. Hence pure juices have more nutritional value than the diluted ones. The redox reaction is better than an acid-base titration since there are additional acids in a juice, but few of them interfere with the oxidation of ascorbic acid by iodine. Generally, the titration method is associated with some interference. Hence quantitative determination of ascorbic acid using highly sensitive instruments such as GC-MS, FTIR, NMR etc which are sensitive, precise and can be used to detect and quantify specific molecule will give better and fast estimation of the ascorbic acid than the titration method. Vitamin C or ascorbic acid is an essential nutrient and a powerful natural antioxidant. The main function of any antioxidant is to boost immunity by scavenging the harmful free oxygen radicals. But what sets vitamin C apart is that it also strengthens the bones, synthesizes collagen and certain neurotransmitters, metabolizes protein, helps fight cancer, and improves iron absorption. But there's a catch. Unfortunately, the human body cannot synthesize vitamin C. And that's the reason you must consume high vitamin C foods to provide your body with the daily required dose of the vitamin, which is 75 mg for women and 90 mg for men per day. The best part is you don't have to just depend on citrus fruits to get your daily dose of vitamin C, there are other foods that are equally rich in this vitamin. Here are 39 vitamin C rich foods (fruits and vegetables) that you should include in your diet. Rose Hips, Green Chili, Guava, Yellow Bell Pepper, Parsley, Red Bell Pepper, Kale, Kiwi, Broccoli, Brussels Sprouts, Cloves, Lambsquarters, Lychee, Mustard Greens, Kohlrabi, Papaya, Strawberries, Orange, Lemon and Lime, Clementine, Pineapple, Cauliflower, Chinese Cabbage, Watercress, Cantaloupe, Cabbage, Collard Greens, Grapefruit, Swiss Chard, Spinach, Gooseberry, Mango, Raspberry and Blackberry, Potato, Peas, Tomatoes, Turnips, Apricots, and Cherries. Vitamin C or ascorbic acid is an electron donor. After donating an electron to a recipient molecule, it becomes ascorbate, which is an essential cofactor for various enzymatic reactions in the body. When there's a deficiency of vitamin C, the lack of cofactor prevents the reactions from taking place, which ultimately leads to weak immunity, weak bones, infections, skin problems, poor wound healing, joint pain, depression, fatigue, inflammation, bleeding gums, scurvy, and anemia. So, it is clear that vitamin C is crucial to maintain a healthy body and strong immunity.

Table 9: Results of Citric Acid Determination

Sample no.	mg citric acid/20mL of diluted juice
Sample 1	30.74
Sample 2	24.40
Sample 3	19.79
Sample 4	23.06
Sample 5	103.75
Sample 6	51.87

From table 9, the order of citric acid content for the fresh juice samples is: sample 5 > sample 6 > sample 4 (5 = orange, 6 = pomegranate and 4 = pineapple). Hence fresh orange juice (citrus fruit) has the highest citric acid content. While the order of citric acid content for the packaged juice samples is: sample 1 > sample 2 > sample 3 (1 = pineapple, 2 = orange and 3 = pomegranate). This order is different from that of the fresh juices. This may be due to the fact that packaged or branded juices usually contain added substances. The values obtained for each sample include all the substances of an acidic nature in the juice that react with NaOH. It so happens, however, that in most fruits the chief substances reacting with NaOH are the organic acids; for this reason, the titratable acidity represents fairly well the organic acid content of a given juice. The predominating organic acid in orange juice is citric acid; but other organic acids, namely, tartaric, malic, beizoic, and succinic, have been reported present. The acid and the base react with one another according to the equation:

$$C_6H_8O_7\ (aq) + 3\ NaOH\ (aq) \longrightarrow C_6H_5O_7Na_3\ (aq) + 3\ H_2O\ (l)$$

Unlike in the ascorbic acid titration where both pure and diluted juice samples were used, the determination of citric acid by titration was feasible only with the diluted samples as the whole 50mL NaOH solution in the burette was consumed with 20mL of pure orange or pineapple juice without a colour change. This is because the fewer amounts of acids in the diluted juice samples will be consumed by the base in less time thereby showing colour change at endpoint. Citric acid is a weak organic acid that has the chemical formula $C_6H_8O_7$. It occurs naturally in citrus fruits. In biochemistry, it is an intermediate in the citric acid cycle, which occurs in the metabolism of all aerobic organisms. More than a million tons of citric acid is manufactured every year. It is used widely as an acidifier, as a flavoring and chelating agent. Citric acid gives pineapples and citrus fruits their sourness, a flavor component so important that soft drink manufacturers add citric acid to many popular beverages. Chefs use crystalline citric acid or sour salt to add tartness to food. On a cellular level, citric acid allows the conversion of glucose to energy. Some individuals react allergically to citric acid, developing hives, a skin rash and breathing problems. Ripe pineapples also contain high levels of malic acid, responsible for sourness in unripe fruit. It is stated that while numerous components of pineapple contribute to its nutritional value, citric acid does not. Citric acid won't hurt you, but it may contribute to acid reflux. However, a statement from Columbia University explains that foods high in citric acid don't actually cause acid reflux, but the acid can make stomach contents more acidic and therefore more irritating to your esophagus. Citric acid is commonly linked to citrus fruits, but it occurs naturally in a variety of fruits. Some fruits contain up to eight percent citric acid, with sour fruits predominantly containing the highest percentages. Citric acid has a tangy and bitter flavor. It is a popular food additive, used to form jams and jellies, stabilize dairy products, preserve meat and provide antioxidant properties to oils and fats. Citric acid is not the same thing as ascorbic acid. It does not prevent fruits and vegetables from turning brown and contains no vitamin C.

CONCLUSIONS

After achieving the aim and objectives of this study, the following conclusions were drawn:
The pH is an important physico-chemical parameter that could cause possible contamination by acid-tolerant pathogens as well as those that may occur in low-acid juices (pH greater than 4.6) if not properly preserved. All juice samples showed the presence of reducing sugar and phosphate which indicated carbohydrate and phosphorus for nutrition. The juice samples in this study contained ascorbic acid with highest value in the fresh pure orange juice sample. It is an essential nutrient and a powerful natural antioxidant. They also contained citric acid with highest value in the fresh orange juice sample. It is an important flavor in many beverages. Between the fresh and packaged juice samples of pineapple, orange, and pomegranate, all the fresh juice samples contain higher concentrations of Cd while two of the packaged juice samples (pineapple and pomegranate) contain higher concentrations of Pb. The packaged orange juice had the least concentrations of both Pb and Cd. All samples had Pb and Cd concentrations that exceeded W.H.O. limits for drinking water. These juices are therefore not safe for consumption. Fe and Zn are essential to the body but the concentrations observed were significantly high. From physical observations in the sampling area during the study, the overall quality of these juices was questionable from the beginning even though the cafes and restaurants in the area serve juices in apparently hygienic conditions in the roadside shops and recreational areas and busy market places. The high levels of heavy metals observed is an indication of possible contamination by these metals which could occur during production stage, harvest and postharvest processing which include transport, storage, and processing of fruit. Thus, the study therefore calls for improvement in the quality of these juices for safe consumption.

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REFERENCES

1. Eka, N., Astuti, Retno, S. and Rohman, A. Validation and quantitative analysis of cadmium and lead in snake fruit by flame atomic absorption spectrophotometry. *International Food Research Journal* ***2012,*** *19 (3): 937-940*

2. Saeed Akhtar, Tariq Ismaila, Muhammad Riaza. Safety assessment of street vended juices in Multan-Pakistan: A study on prevalence levels of trace elements. *International Journal of Food and Allied Sciences* ***2015,*** *1(1): 1-8*

3. Anila Anwar, Talat Mahmood, Qamar- Ui- Haque, et al. Heavy metals in fruit juices in different packing material. *fuuast j. biol.* ***2014,*** *4(2): 191-194*

4. Heghedűş-Mîndru Ramona Cristina, Heghedűş-Mîndru Gabriel, Negrea Petru, Şumălan Radu, Negrea Adina¸ Ştef Ducu. The monitoring of mineral elements content in fruit purchased in supermarkets and food markets in Timisoara, Romania. *Annals of Agricultural and Environmental Medicine* ***2014,*** *21(1): 98–105*

5. Z. Krejpcio1, S. Sionkowski, J. Bartela. Safety of Fresh Fruits and Juices Available on the Polish Market as Determined by Heavy Metal Residues. *Polish Journal of Environmental Studies****2005,*** *14(6): 877-881*

6. Lyudmila Melnyk, Oleksandr Bessarab, Svitlana Matko and Myroslav Malovanyy. Adsorption of heavy metals ions from liquid media by palygorskite. *chemistry & chemical technology* ***2015,*** *9(4): 467 – 470*

7. Kalagbor Ihesinachi and Diri Eresiya. Evaluation of heavy metals in orange, pineapple, avocado pear and pawpaw from a farm in Kaani, Bori, Rivers State Nigeria. *International Research Journal of Public and Environmental Health* ***2014,*** *1(4): 87-94*

8. Zeid A. Al Othman. Lead Contamination in Selected Foods from Riyadh City Market and Estimation of the Daily Intake. *Molecules* ***2010,*** *15(1): 7482-7497*

9. Girma W. Woyessa, Solomon B. Kassa, Ephrem G. Demissie, B.B.L. Srivastava. Determination of the level of some trace and heavy metals in some soft drinks of Ethiopia. *Int. J. Curr.Res.Chem.Pharma.Sci.* ***2015,*** *2(3): 84–88*

10. Prakash P , Rajan G, Elavarasi V, Poornima Priyadharsani T. K, Thamaraiselvi K. Assessment of Heavy Metal Contamination (Nickel and Arsenic) using GF-AAS in Local Brand Chocolates and Candies from Tiruchirappalli, India. *International Journal of Computer Applications (IJCA)* ***2014,*** *(0975 – 8887): 25 – 28*

11. Ithar Kamil Al-Mayaly. Determination of some heavy metals in some artificial fruit juices in iraqi local markets. *Int. J. Res. Dev. Pharm. L. Sci.* ***2013,*** *2(4): 507-510*

12. Sobia Akram, Rahila Najam, Ghazala H. Rizwani and Syed Atif Abbas. Determination of heavy metal contents by atomic absorption spectroscopy (AAS) in some medicinal plants from Pakistani and Malaysian origin. *Pak. J. Pharm. Sci.*, ***2015,*** *28(5):1781-1787*

13. Itodo U. Adams and Itodo U. Happiness. Quantitative specification of potentially toxic metals in expired canned tomatoes found in village markets**.** *Nature and Science.* ***2010,*** *8(4):54-59*

14. Adepoju-Bello AA, Oguntibeju O.O, Onuegbu MT, Ayoola GAA and Coker HAB. Analysis of selected metallic impurities in soft drinks marketed in Lagos, Nigeria. *African Journal of Biotechnology* ***2012,*** *11(20): 4676-4680*

15. A. I. Ajai, S. S. Ochigbo, Z. Abdullahi, and P. I. Anigboro. Determination of Trace Metals and Essential Minerals in Selected Fruit Juices in Minna, Nigeria. *International Journal of Food Science* ***2014,*** *1(1): 1–5*

16. S.M. Farid and M.A. Enani. Levels of trace elements in commercial fruit juices in Jeddah, Saudi Arabia. *Medical Journal of Islamic World Academy of Sciences* ***2010,*** *18(1): 31-38*

17. Ogunlana O.O., Ogunlana O.E., Akinsanya A.E. and Ologbenla O.O. Heavy Metal Analysis of Selected Soft Drinks in Nigeria. *Journal of global biosciences* ***2015,*** *4(2): 1335 – 1338*

18.Engwa Azeh Godwill, Ihekwoaba Cynthia Jane, Ilo Uchenna Scholasticab,Unaegbu Marcellusa, Ayuk L. Eugene, Osuji Amarachukwu Gloria. Determination of some soft drink constituents and contamination by some heavy metals in Nigeria. *Toxicology Reports* ***2015,*** *2(1): 384–390*

19. Kamal Rai Aneja, Romika Dhiman, Neeraj Kumar Aggarwal, Vikas Kumar, and Manpreeet Kaur. Microbes Associated with Freshly Prepared Juices of Citrus and Carrots. *International Journal of Food Science* ***2014,*** *14 (1):1 – 2*