# QUANTIFICATION OF TOTAL IRON CONTENT OF *MALUS PUMILLA* (APPLE) USING SPECTROPHOTOMETRIC METHOD

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**Abstract**

Present study comprises on study of commonly available fruit *Malus Pumilla (*apple). The moisture content is found to be 81±2%. Aqueous and acidic mediums (0.1M HCl) were used for the analysis of total dissolve (TDS) and un-dissolved solid (UDS). Results revealed that maximum TDS in aqueous medium was 8.2% while in acidic medium it was 70.7%. So acidic medium is more favorable for extracting TDS. The analysis of total metal content through EDTA titration revealed that $HNO_3$ is the best medium and can extract out up to $9.6x10^{-2}$mmoles/g of metal. Spectrophotometric method was used to determine the total iron content in digesting samples. Results reveal that the iron content of an apple is 0.033 to 0.09 ppm which is within the tolerance limit.

## Introduction

Apple *Malus Pumilla* is a pomaceous fruit belong to the family Rosaceae. It is one of the most widely cultivated tree fruits. The different varieties available in our country are Amri, Kandhari, Kulu, Mashadi, Golden delicious and Red delicious etc. This study is to explore the amount of total metals dissolved in the acidic environment of the stomach and also measure the total iron content digest in the stomach. The carbohydrates give sweetness to the appeal. The carbohydrates are fructose, glucose, sucrose and cellulose and Pectic substances. It contains macro elements sodium, potassium, magnesium, calcium and phosphorous while the micro elements of the fruit are iron, zinc, copper and manganese (Zia-ur-Rehman *et al.* 2002).

Apples are a significant dietary source of antioxidants and have been associated with a reduced risk of chronic disease (Akazone, 2004). Boyer and Liu (2003,2004) found apple to have the second highest level of antioxidant activity, compared to other commonly consumed fruits (Boyer and Liu, 2003/04). It is also a significant source of antioxidant, therefore it is widely used in different aging creams, scrubs and for medicinal purposes. Average sized apple contain 6 milligram of vitamin C, due to the presence of vitamin C and high amount of iron human are safe from anemia. Ref. Iron is an absolute requirement for most forms of life, including humans, most bacterial species. Plants and animals and it can be found in a wide variety of food sources and apple is one of them (Fatima, 2004). For the analysis of iron many methods are available like spectrophotometry (Rajashree1 *et al.*, 2012), atomic absorption spectrometry (Fatima, 2005), cathodic striping Voltammetry (Croot and Johansson, 2000) and fluorimetry (Pulido-Tofiño, 2000) etc. Spectrophotometry is most widely used due to its simplicity. This technique involved the use of ligands which selectively bind to metal and produce a colored complex. Different spectrophotometric methods are available for analyzing the iron content in biological samples (Jafarian-Dehkordi *et al.*, 2008, Kenjiro *et al.* 1986, Ahmed and Roy, 2009). However in the present study an easy and economical method is applied for Fe (III) analysis in the ash of locally available apples. Thiocyanate form a red colored water-soluble complexes in acidic solution with Iron (III). This reaction has been widely used as a conventional method for the spectrophotometric determination of Fe (III) (Sandell, 1959). Beside this moisture content, TDS, UDS and total metal content also determined.

The present research work comprises on the study of different parameters like moisture content, percentage of TDS and UDS in aqueous and acid environment and also determined the total metal content by complexometric titration.

In the present study

## Materials and Methods

**Sample collection:** From local market of Karachi-Pakistan fruits of *Malus Pumilla* (Apple) were purchased. They were washed properly to remove dust and other material before analysis.

Analytical grade Chemicals (Merck and Sigma) were used without further purification. Glassware was washed properly and then distilled deionized water was used for rinsing. For preparation of different solution distilled deionized water was used.

**Instrumentation:** The total iron content was determined at Vis-7220 spectrophotometer at $_{max}$ (480nm). The cell path length was 1cm.

**Moisture content:** 15 hours were required for the removal of moisture content with skin for 5 to 6 grams of apple (at 105$^{o}$C).

**Sample preparation:**
**Soaked samples:** Apple was clean and chopped properly and then after accurately weighing soaked in deionized water for different time intervals (1 to 6 Hours). This solution was filtered and then made up to the required volume. The same procedure was repeated for other mediums (0.1M HCl and 0.1M $HNO_3$).

**Digestion of apple:** Accurately weighed 50-60 gram of pulp was made moisture free and then Concentrated $HNO_3$ (5mL of 69.5%) was added and heated to dryness. 5mL of (Conc.) $HNO_3$ and 2mL $H_2O_2$ was again added into it and heated to get clear solution and then filtered and made up to distilled deionized water up to the required volume (named as sample 'W').
The same procedure was repeated except the solution was made up another two mediums i.e. HCl (0.1M) and $HNO_3$ (0.1M). These samples were analyzed for total iron content using visible spectroscopy and named as 'sample H' and 'sample N'.

**EDTA titration of soaking samples:** For an EDTA titration pH of soaked sample was maintained up to 10 (ammonia/ammonium chloride) buffers and then titrated against EDTA(sodium salt) using Eriochrome Block T. At the end point color changes to green. EDTA titration was performed in different soaked samples at different time intervals (Vogel,1989). Preparation of standards:

**Preparation of standards and blank:** Iron standards of $FeCl_3.6H_2O$ (acidified with HCl) were prepared from a stock of 0.001M solution. For this purpose standards from 1 to $6x10^{-5}$M were prepared. For the preparation of different standards required volume of iron was transferred in a 25mL volumetric flask and then 5mL of 2M solution of KSCN was added to obtain red colored solution and absorbance were recorded at 480nm (Vogel,1989).
Blank of KSCN+ water/HCl/$HNO_3$ was used for calibration of the instrument.

**Spectrophotometric determination of iron in digesting samples:** 20mL of ammonia was added in 10mL of ash sample to precipitate out the iron content of the sample. These ppt were filtered and as a result of which iron precipitates out as $Fe(OH)_3$. These precipitates were dissolved with HCl (Conc.) in a 25mL volumetric flask. For chelation 5mL of KSCN was added and made the solution up to mark with distilled deionized water. The red color is obtained due to chelation which gave maximum absorbance at 480nm. This method is adopted for the removal of interfering metals of iron (Sandell, 1959).

**Results and Discussion**

Locally available apple contains a huge amount of moisture i.e. 81±2% (Fig. 1) So it easily helps to fulfill the moisture content of the consumer. The percentage of TDS and UDS was determined in aqueous and acidic extract (0.1M HCl). Results show that in both mediums % TDS increases with time. Maximum %TDS was obtained within 6 hours. In the water it was 8.2% while in acidic medium it was 70.7% (Fig. 2a & 2b). On the other hand %UDS decreases with respect to time in both the mediums. Undissolved materials were last up to 6 hours in water i.e. 1.5% while in acidic medium it was 1.9% (Fig. 2a & 2b). Results indicate that the acid (0.1M HCl) can extract out more TDS than water (Fig. 2a-2c). It is concluded that a large portion of apple is dissolved in the acidic environment of the stomach.

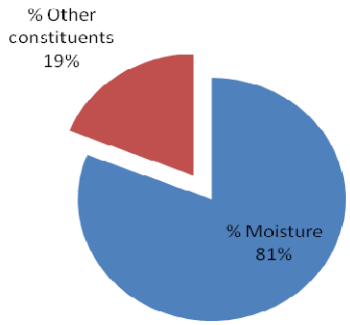


**Fig.1. Moisture content and other constituent of *Malus Pumilla* (Apple).**

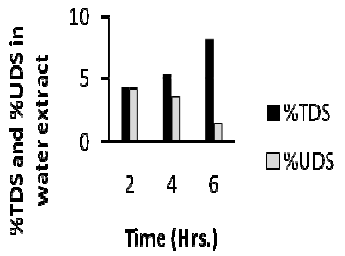


**Fig. 2a. Effect of time on %TDS and %UDS of *Malus Pumilla* (Apple) in aqueous extract.**

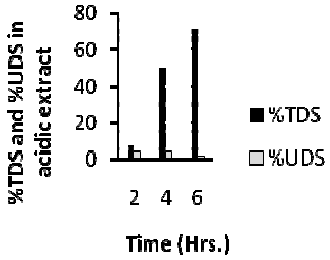


**Fig. 2b. Effect of time on %TDS and %UDS of *Malus Pumilla* (Apple) in acidic extract.**

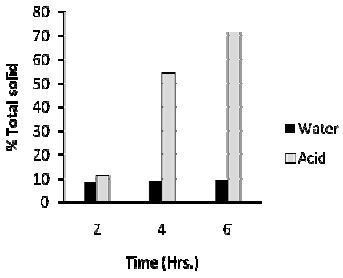


**Fig. 2c. Comparison of effect of time on %Total solid of *Malus Pumilla* (Apple) in acidic (0.1M HCl) and aqueous extract.**

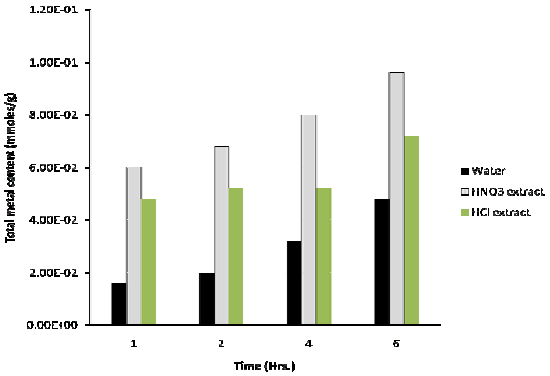


**Fig. 3. Effect of time on total metal content of different extracts of *Malus Pumilla* (Apple).**

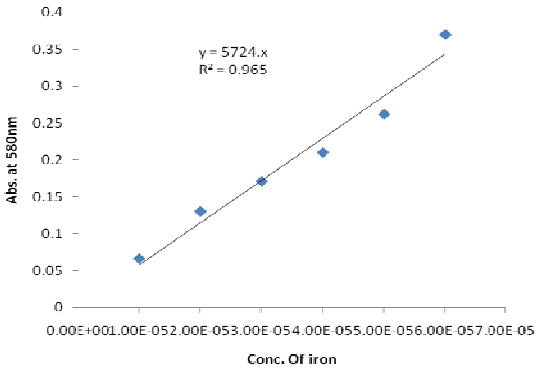


**Fig. 4. Calibration curve for Fe-SCN.**

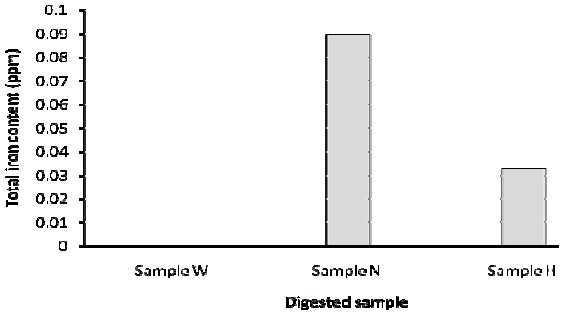


**Fig. 5. Total iron content in different digested samples of *Malus Pumilla* (Apple).**

**Table 1. Total iron in different digested samples of *Malus Pumilla* (Apple).**

| Digested Sample | Conc. (ppm) of total iron |
|---|---|
| Sample W | ----- |
| Sample N | 0.09 |
| Sample H | 0.03 |
| *Recommended daily intake of iron (mg/70Kg/body weight) | 8-18 (mg/recommended dose/day) |

* (Institute of Medicine, 2001)

Results revealed that water can extract least ($4.8x10^{-2}$mmoles/g) while $HNO_3$ can extract the maximum amount ($9.6x10^{-2}$mmoles/g) of total metal content. The ability of $HNO_3$ can be due to its strong oxidizing property. HCl can extract out medium amount ($7.2x10^{-2}$ mmoles/g) of total metal content as compared to both of the mediums within 6 hours (Fig. 3).

**Total iron content:** total iron content was determined in digesting sample by using a economic spectrophotometric method i.e. Fe-SCN method. When whole iron converts into its highest oxidation state in terms of Fe(III) can be analyzed at 480nm. Different concentration solutions were used for analyzing of iron content (Fig. 4). Results revealed that the ash which was made up in $HNO_3$ (Sample N) provide maximum iron content i.e. 0.09ppm as compare to HCl (Sample H) 0.033ppm. (Fig. 5, Table 1). The iron content of apple is found to be 0.03-0.09ppm. The reported iron content of an apple is 1.8-6.0ppm in different varieties of apple of different districts of Pakistan. The daily recommended dose for iron is 08 to 18 mg (Elite Whitney *et al*.). The present study shows that apples can be helpful for obtaining iron content in our body (Table 1).

## Conclusion

Fruits are the natural source of essential trace elements. The present study concerned with the daily intake of an apple can easily provide 0.03 to 0.09ppm of iron and also a large amount of moisture content (i.e. 81±2%) can fulfill the water content of our body. Results of %TDS and %UDS in aqueous and acid environment show that the environment of the stomach is very favorable for obtaining apple benefits in terms of TDS and UDS and it also indicates that the polar components of the fruit required very short time in the acidic environment of the stomach.

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