Comparative insight into the reducing effect of vitamin C on the iron content in the cabbage irrigated with fresh and sewage water

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Abstract

This research work is based on the comparative study of vitamin C and Fe-content present in cabbage grown with different water regimes used for irrigation purposes as well as reducing effect of vitamin C on Fe-content because of the increasing consumption of cabbage across the world. The concentration of vitamin C was estimated by two methods; iodimetry and indophenol method while1, 10-orthophenonthrolein (OPT) and potassium thiocyanate (KSCN) methods were applied for total iron and ferric ion estimation, respectively. The experimental results unambiguously demonstrate that the concentration of vitamin C was observed in higher concentration in the cabbage grown in sewage water than in cabbage grown in fresh water. However, the amount of iron observed was comparatively higher in the cabbage grown in freshwater (CK). The estimated amount of Fe^{+2} in the cabbage sample was observed that is; vitamin C plays an important role in the reduction of ferric into ferrous in the cabbage.

Keywords: Vitamin C, Iron estimation, cabbage analysis, reduction of iron, water irrigation.

1. Introduction:

In a biological system, “an antioxidant is a species that would significantly delay or inhibit the oxidation of the substrate”. The main form of vitamin C, in which it exists, is an L-ascorbic acid (L-threo-hex-2-enono-1,4-lactone, ascorbate) (Reddy, 2017). Some oxidizing agents such as potassium permanganate, methylene blue, phenol indophenol, and ferric salt are widely used in the determination of vitamin C, as they oxidize the ascorbic acid to give dehydroascorbic acid ($C_6H_6O_6$) (Reddy, 2017; Jose;, 2011). Different methods are used for the determination of vitamin C such as cyclic voltammetry, titration with N-bromosuccinimide (Ogunlesi, 2010), 2, 6-chlorophenols indophenol titration method (Reddy, 2017) and HPLC method (Cenek et al, 2018).Vitamin C is soluble in water and extensively distributed in fresh fruits and vegetables. It is present in fruits such as orange, pineapple, raspberries, and cherries. Green leafy vegetables like tomatoes, broccoli, green and red peppers, cauliflower, and cabbage also contain sufficient vitamin C(Naidu, 2003). To fulfil the vitamin C requirements, different beverages, chemicals, drugs, and other foods are incorporated by vitamin C (Ojukwu, 2017).

Vitamin C is needed for the formation of some intercellular substances which play a role in cell interconnection in the tissues of certain organs of the body (Ajibade,1997).Some studies show the role of vitamin C in collagen formation, iron absorption, improvement of the immune system (Wintergerst, 2006), and free radicals response (Reddy, 2017).Deficiency of vitamin C in the body may cause diseases such as loosening of tooth tissue, weak bones, periosteal bleeding (Hodges, 1969), cognitive impairment to the child, scurvy, gum disease, and unhealthy skin (Reddy, 2017).

The heating and cooking of vegetables can readily affect the loss of vitamin C. It is thermolabile and susceptible to oxidation. These properties promote vitamin C more susceptible to degradation (Joy, 2007).Even though some studies have reported the boiling of vegetables cannot affect the destruction of vitamin C (Afshan, 2016). Humans, primates, guinea pigs, red vented bulbul, and some other animals cannot synthesize vitamin C because human cells are unable to convert L-gulono-g-lactone into vitamin C in the presence of catalyst gulonolactone oxidase enzyme during the biosynthesis of vitamin C (Reddy, 2017; Ojukwu, 2017).

Cabbage is an excellent source of vitamin C also containing some vitamins B, cabbage supplies some potassium and calcium to the diet. It contains 8.4 kilocalories/100gm as raw and 23.2 kilocalories/100gm when cooked (Haque, 2006).Citrus fruits are widely used by industries to bean important source of vitamin C and flavonoids. (Rio, 1997). Lemon is one of the fruits that grow well in the tropical and semi-tropical regions, but sub-tropical regions are not considered compatible with their growth (Mansfield, 2003). Lemon, being a good source of vitamin C, helps the human body to form connective tissues, bones, and different blood vessels. But the most important role is to play as an antioxidant, which improves the body’s defence system against any kind of reactive oxygen species (ROS) and free radicals (Okieiet, 2009).

(Koji et al, 2011) Reported haemoglobin is significantly present in lemon juice which has a substantial impact on iron bioavailability. Iron plays a considerable role in numerous biochemical functions in animals and plants. In particular, iron has directly associated with the haemoglobin formation and functioning of other organs of the body (Iffat, 2019). Elemental iron and heme or non-heme iron are basic components of dietary iron (Zhu, 2010). Vegetarians depend on non-heme sources of iron, which results in low susceptibility to low iron intake because heme sources contain a higher amount of iron (Radhika Narain, 2005). According to (Anthoney Swamy, 2016), while cooking vegetables, Fe content is altered. Iron is one of the essential components which maintains the level of haemoglobin in human beings (Iqbal, 2019).Our previous experiment reported that Fe accumulated in the root, leaf, and stem of vegetables were found to be higher as compared to other metals (Faiza, 2016). It is also reported by (Hurrell, 1997)that iron is needed for some type of enzyme which is required in electrochemical and redox reaction reactions in the human body. One of the WHO reports shows that 50% of women and 42% of children suffer the disease, iron deficiency anaemia across the world(WHO, 2017).

There is a need to measure the amount of iron and vitamin C simultaneously in vegetables to estimate which type of vegetable sources is rich in vitamin C and iron, so that they may be consumed to fulfil the daily requirement of vitamin C and iron. Almost all previous studies have been carried out to estimate vitamin C and iron content in cabbage, regardless of the water irrigation effect. Based on these facts and considerations, the present study was carried out to estimate and compare the impact of different water irrigation systems on the uptake of the iron and vitamin C content and the considerable effect of the vitamin C on the reduction of iron in cabbage.

2. Materials and Methods:

2.1 Analysis of samples:

2.1.1. Determination of vitamin C by Indophenol Method:

Prepared a 0.0048M (0.243gm/250mL) solution of indophenol in distilled water and kept on stirrer at 50'C for 20 minutes. Standardized the indophenol solution against a standard solution [15mL oxalic acid, 1mL sodium acetate, 15 mL distilled water, and 1mL vitamin C solution].The sample extract was then standardized against the standard indophenol solution. All works were performed in triplicate.

2.2.2. Determination of vitamin C by Iodometry:

The vitamin C content was determined by the classical mode of examination based on iodometry in which sample extracts were standardized with standard iodine solution in the presence of the starch indicator. All analyses were done in triplicate.

2.2.3. Collection, pretreatment, drying, and digestion of samples for iron estimation:

The Collection, pretreatment, and digestion of samples are the most important steps in chemical analysis. Cabbage samples grown in sewage water and freshwater were collected in a local vegetable market of Malir Karachi and an open street market in Khuzdar Baluchistan, Pakistan respectively. All collected samples were properly cleaned and rinsed with distilled water for the removal of soil particles. Moisture was removed using a hot air oven at 110-120°C for 5-6 hours. The cabbage samples were cut into small pieces; the samples were homogenized into two bowls. Both the bowls were kept in the oven at 100˚C. Dried samples (black colour) were shredded properly to form a fine powder. All samples were prepared and analyzed in triplicates. Six crucibles were taken, labelled as K1, K2, and K3 for the sample taken from Khuzdar while M1, M2, and M3 for the sample taken from Malir. Accurately 3g of dried sample was weighed and placed in a furnace at 550°C for 12 hours.

All samples were converted to ash with the exception of two samples K1 and K3, so these samples were kept for further 6 hours in the furnace. Once the grey or white ash was obtained 4mL of 4M HCl was added to dissolve the samples. Samples were then transferred into test tubes and then centrifuged in a centrifuge machine for 3 minutes in order to settle the un-dissolved particles. Filtered the solution through filter paper and washed the filter paper with a 4M HCl. Finally, they were transferred into 25mL volumetric flasks by raising volume up to the mark with HCl solution. Now we have six 25mL volumetric flasks for six samples. The samples were kept overnight before complexation.

2.2.4. Determination of total Iron:

1,10-orthophenonthrolein (OPT) method was used for the estimation of total iron. Hydroxylamine hydrochloride was used as a reducing agent to reduce all kinds of iron species into ferrous form. OPT was the complexing agent to form Fe (II)-opt complex (orange-red). Fe(II)–OPT is stable in the pH range of 5 to 9. Hence the pH is adjusted by adding sodium acetate buffer. All parameters were optimized for complexation. The volume of reducing agent and buffer solution was set at 2ml and 4ml respectively, 30 minutes were found to be the proper time for complexation, the volume of opt was fixed on 2.0mL throughout the experiment. Prior to complexation, the digested samples of cabbage were kept overnight after digestion and then dissolved in HCl. All the works were performed in triplicates. Absorbance was determined by using a UV-Visible spectrophotometer (Jenway 6310) at 525nm. The total iron was estimated using the calibration curve method. For the calibration curve method, a standard solution of Fe (II) of different concentrations was used after treatment with reagents as mentioned above.

2.2.5. Determination of ferric form of Iron:

For the KSCN method, potassium thiocyanate was used as a complexing agent to form theFe (III)-SCN complex (blood red). The absorbance was determined by a UV-Visible spectrophotometer (Jenway 6310) and the maximum absorbance was set at 458nm.Fe (III) was determined by the slope-point method.The ferrous ion was determined by the difference between total iron and ferric form of iron.

1. Results and discussion:

3.1 Estimation of vitamin C in the cabbage and lemon sample:

Cabbage is the most important and is found primarily in locally available leafy vegetables grown in soils irrigated with tap and sewage water. The present study focused on reducing effect of vitamin Con the iron content in cabbage samples. Chemically vitamin C is a reducing agent and weak acid. Vitamin C reacts with oxidizing agents such as iodine and indophenol and itself changed into reduced form as**eq. 1** given below**:**

$$\underset{\text{Ascorbic acid}}{C_6H_8O_6} \underset{\text{Reduction}}{\overset{\text{Oxidation}}{\rightleftharpoons}} \underset{\text{dehydroascorbic acid}}{C_6H_6O_6} \quad \textit{Eq. 1}$$

Quantitatively and qualitatively, vitamin C was analyzed through two methods: the volumetric and the indophenol method. The reaction between vitamin C and indophenol is given as; [Gideon, 2014].

$$H_2C_6H_6O_6 + HC_{12}H_6Cl_2O_2N \rightarrow C_6H_6O_6 + HC_{12}H_8Cl_2O_2N \quad \textit{Eq. 2}$$

Vitamin C2,6-dichloroindophenoloxidized vitaminCreduced 2,6-dichlorophenol

(Colorless) (red) (brown) (colorless)

While the reaction between vitamin C and iodine is given as; [Cesar, 1999].

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2H^+ + 2I^- \quad \textit{Eq. 3}$$

In Malir Karachi (MK), vegetables are cultivated in sewage water and in Khuzdar Quetta (CK), vegetables are cultivated in freshwater. The results are given in tables 1 and 2 denote the amount of vitamin C in the cabbage by iodometry and indophenol method, respectively. A higher amount of vitamin C was estimated in the cabbage grown in sewage water (CM) as compared to freshwater (CK). Because the sewage water can impact diverse effects on the properties of the soil; consequently, the uptake of minerals is changed. To compare the effect of irrigation, the same techniques were used for lemon extract, which was also collected from the same irrigation area as the cabbage sample. The trend of result for lemon extract was similar to as observed for cabbage in same water of irrigation.

The results of these two methods were almost in good agreement. While comparing the amount of vitamin C in lemon and cabbage, it is clear from tables 1 and 2, the lemon sample has a significant amount of vitamin C as compared to the cabbage sample. Because many kinds of literature have shown lemon is one of the good sources of vitamin C.We can see in all reported results from tables 1 and2, the iodometric method has less precision as compared to the indophenol method in the results may be because of instrumental or observational error.

The Indophenol method has precise results, which may show its accuracy as well. Secondly, iodometry is a time-consuming experiment. We need to standardize three different solutions and handling the iodine solution in "the dark environment" is another challenge.

The third factor is the different reactivity, enthalpy of reaction, ambient condition, and reaction mechanisms of vitamin C with iodine and with that of indophenol may interfere in the observations and results. Because the nature of the reaction of eq 2 differs from the reactive nature of eq. 3.

In terms of the amount of vitamin C in cabbage, we compare our results with other studies.[Vogtmann, 1993] and [Bavec, 2010] have reported about green manure that raises the amount of vitamin C content in cabbage. Comparing our results with one of the reports on lemon juice [Aurelia et. all], our experimental amount by iodometryis 0.037 g/50mL and 0.052 g/50mL while by indophenol method are 0.035 g/50mL and 0.047g/50mL, which are twice the reported value of respective research work (0.0176 g/50mL).The different results of vitamin C in the cabbage sample in our work and other reported literature maybe because of the different vegetarian periods, the water of irrigation, and the geographical situation of different countries. Vitamin C decreased as the vegetable ripened [Mario, 2019], maybe our vegetable sample was on the ripening stage, so that we found a comparatively higher amount of vitamin C in these two samples. The graphic report in tables 1 and 2 is presented in Fig 1 for further comparison. As different countries have different cultivation systems and different kinds of manure used in the cultivation. These factors contribute to the variation in vitamin C in cabbage.

The previous report [Anita, 2010] shows that vitamin C is higher in plants at wastewater irrigated sites. Lemon was analyzed just to look at the effect of water irrigation on the amount of vitamin C.

3.2 Estimation of iron species in the cabbage sample:

The total iron and ferric(Fe^{+3}) iron concentrations were determined by the Iron OPT method and the thiocyanate method, respectively.1,10-orthophenonthrolein (o-phen) is a nitrogenous heterocyclic tricyclic compound that reacts with different heavy metals such as iron, nickel, and gold to form complexes.

$$\mathbf{Fe^{+2} + 3\text{-o-phen} \rightleftharpoons Fe(o\text{-}phen)_3^{2+}} \quad \textit{Eq. 4}$$

The Fe-OPT complex formation is given by the following equation:

$$\mathbf{2Fe^{+3} + 2NH_2OH.HCl + 2OH^- \rightleftharpoons 2Fe^{2+} + N_2 + 4H_2O + H^+ + Cl^-} \quad \textit{Eq. 5}$$

The above reaction is an equilibrium reaction, a mild reducing agent drive it more than 99.99% forward. Using prepared solutions, the absorbance vs concentration graph was plotted as shown in fig 2.For the KSCN method, potassium thiocyanate was used as a complexing agent and it gave theblood-red Fe (III)-SCN complex, so its amount was also set as a function of time. The reaction between potassium thiocyanate and iron (III) is given as:

$$\mathbf{Fe^{+3}(aq) + 6SCN^-(aq) \rightarrow [Fe(SCN)_6]^{3-}(aq)} \quad \textit{Eq. 6}$$

5mL KSCN was found to be sufficient for complete complexation. Proper time was found to be 30 minutes which was required for proper complexation. If the pH is elevated, the ferrous will be re-oxidized to ferric. Therefore, a buffer solution of sodium acetate or ammonia may also be used to maintain the pH (5 to 9) of the system. The absorbance of the standard complex $\mathbf{[Fe(SCN)_6]^{3-}}$and the sample complex, respectively at 458nm, are given in tables 3and 4.The concentration of Fe^{+3} was estimated by using the single-point method as the formula given in the supplementary information.

The formula of the Slope point method:

$$\frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} = \frac{\text{Concentration of unknown}}{\text{Concentration of standard}}$$

The analyzed data in table 3, shows a higher concentration of Fe^{+2} than Fe^{+3} for both CK (irrigated with freshwater) and CM (irrigated with sewage water). As we have also estimated a higher amount of vitamin C in CM than CK. As a result, vitamin C plays an important role in the reduction of ferric into ferrous in cabbage irrigated with the sewage water. Data are given in table 3 as well as supplementary table 3, showing a higher concentration of total iron for CK as compared to CM. This result is in good agreement with the work we reported earlier [Iffat, 2019].

Although our current results, presented in table 3, for the concentration ratio Fe^{+2} to Fe^{+3} are not compatible with our previous work (only for freshwater). Here, we acknowledge that CK1 and CK3 were not combusted well in the first round of the combustion, so we burnt them again for one more time overnight in a furnace. So, it is clear from table 3, that the concentration of total iron and Fe^{+3} is slightly higher for CK1 and CK3 as compared to CK2 (which was combusted in the first round).

But the higher ratio of Fe^{+2} in freshwater than Fe^{+3} may be some problems for the KCN method; (i) incomplete digestion of the samples, incomplete burning of the samples etc., so in this way, the absorbance of FeSCN was not that high. Consequently, concentration may also be lower than what we expected. (ii) Previous study [Y.-H. Peggy] reported that the reducing ability of vitamin C on iron content depends upon the pH. The optimum pH is recorded as 5 for the reduction of Fe^{+3} by vitamin C. By increasing the pH, the reducing ability of the vitamin C becomes slow, i.e., above pH 5 the effectiveness of vitamin C is diminished. (iii) We estimated the total iron by the calibration curve method while the ferric form of iron was estimated by the slope-point method. These two methods of calculation may have interfered in the concentration values. Despite this, the concentration of CK1 and CK3 is slightly below our expectations but more accurate than that of CK2. Because the estimation of Fe^{+2} is merely based on the calculation and hence, no influence of any experimental handling on it.

Figure 3 presents the pictorial form of table 3, is given in fig 3. A comparatively higher amount of vitamin C in cabbage irrigated with sewage water caused the formation of Fe^{+2}.

3.3 Chemistry and formation mechanism of vitamin C and Fe^{+2} in the Cabbage:

Although, vitamin C occurs naturally in the cabbage sample. During the OPT method, the pH of the experimental system was maintained between 5 and 9 by using sodium acetate. So, it minimizes the reducing ability of vitamin C to some extent. It will be some what risky to say about the actual pH inside the vegetable during its irrigation. Thus, the rate of reaction between vitamin C and iron will depend on the actual pH conditions inside the vegetables. Vitamin C is not only the responsible reductant for an iron reduction in the vegetables, but also phenolic groups, thiol contents, and many other antioxidants may contribute their activity for the reduction of iron. Sewage water irrigation favourably impacts the physiological and growth characteristic of plants. Vitamin C scavenges free radicals which are generated by heavy metals. Heavy metals in plants stimulate the formation of reactive oxygen species. In order to tolerate these stress conditions, plants produce more secondary metabolites, enzymatic and non-enzymatic antioxidants to scavenge reactive oxygen species. In this way, vegetables develop a strategy to protect against the toxicity of heavy metals [Anita, 2010]. Consequently, the vitamin C produced plays an important role in the reduction of the ferric form of iron into ferrous form. The vitamin C reduces Fe^{+3} as follows:

$$H_2C_6H_6O_6 + 2Fe^{+3} \rightarrow C_6H_6O_6 + 2Fe^{+2} + 2H^{+} \quad \textit{Eq. 7}$$

Conclusion:

This study was carried out to better understand the amount of vitamin C and Fe-content in the cabbage grown under irrigation conditions. This research quantifies the impact of freshwater and sewage water irrigation on the uptake of vitamin C and iron species. It was noticed that the cabbage is grown in fresh water and sewage water have a remarkable aggregate of iron and vitamin C contents, respectively. In addition, another objective of this study was to compare the results of different techniques, these results were roughly in good agreement with one other. The results showed that either the irrigation with freshwater accumulates a high amount of iron in the soil, and it can also be transferred into vegetables growing on that soil or all irons are naturally occurring inside the cabbage. This outcome demonstrates the direct relationship between vitamin C and the reduced form of iron in cabbage.

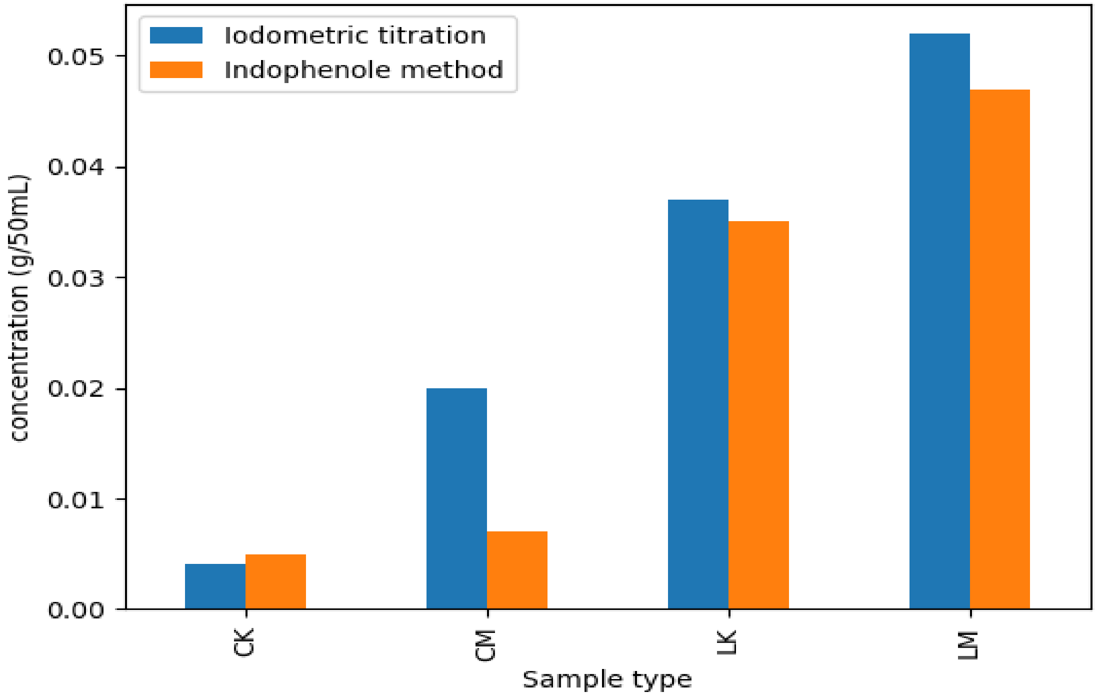

Fig. 1. Amount of ascorbic acid by iodometry and indophenol method

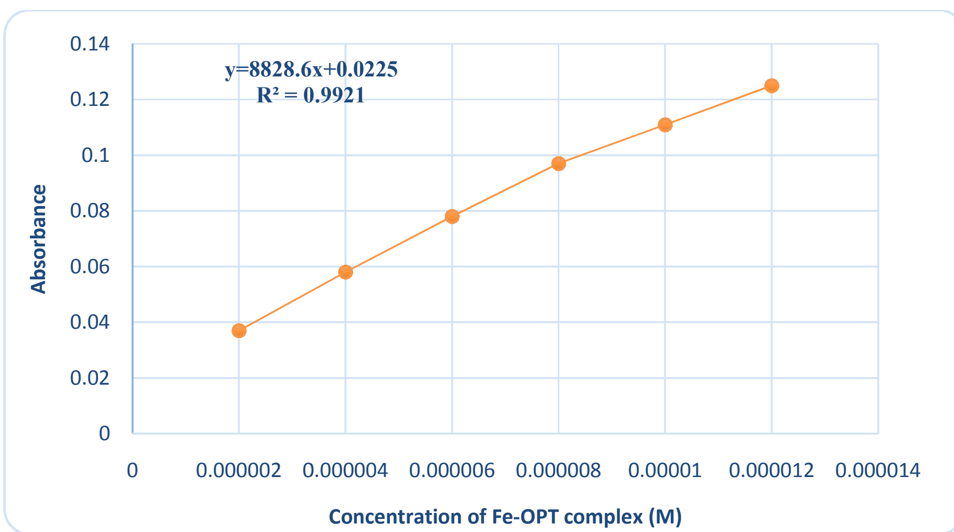

Fig. 2. Calibration curve for Fe-orthophenanthroline (Fe-OPT) complex.

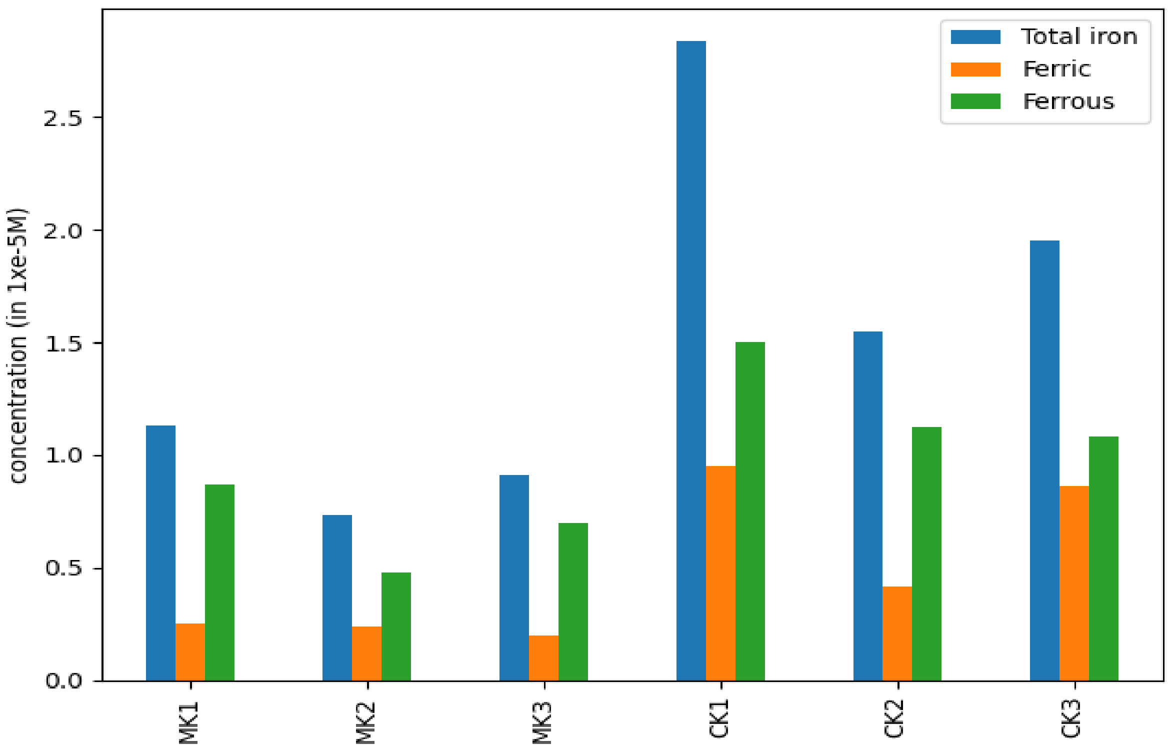

Fig. 3. Comparison of total iron, ferric and ferrous in cabbage

Table 1.The concentration of vitamin C by Iodometry. CK= Cabbage Khuzdar, CM= Cabbage Malir, for 20mL cabbage extract. While LK= Lemon Khuzdar, LM, Lemon Malir, for5mL lemon extract.

Sample code	Volume of iodine solution (mL)	Concentration of vitamin C (M)	Amount(in g/50mL)
CK	10.6	0.00053	0.004
CM	46.0	0.00230	0.020
LK	21.3	0.00426	0.037
LM	30.1	0.0062	0.052

Table 2. Concentration of vitamin C by Indophenol method. CK= Cabbage Khuzdar, CM= Cabbage Malir, for 10mL cabbage extract. While LK= Lemon Khuzdar, LM, Lemon Malir, for1mL lemon extract.

Sample code	Volume of indophenol (mL)	Concentration of vitamin C (M)	Amount(in g/50mL)
CK	6.0	0.000588	0.005
CM	8.4	0.000823	0.007
LK	4.1	0.00401	0.035
LM	5.5	0.00539	0.047

Table 3. Concentration of total iron, Ferric and Ferrous.

Sr. No	Sample code	Concentration of total iron (M)	Concentration of ferric (M)	Concentrationof ferrous (M)
1.	CM1	1.13×10^{-5}	2.53×10^{-6}	8.77×10^{-6}
2.	CM2	7.30×10^{-6}	2.48×10^{-6}	4.82×10^{-6}
3.	CM3	9.10×10^{-6}	2.08×10^{-6}	7.02×10^{-6}
4.	CK1	2.84×10^{-5}	$9.50 X 10^{-6}$	1.50×10^{-5}
5.	CK2	1.55×10^{-5}	4.24×10^{-6}	1.12×10^{-5}
6.	CK3	1.95×10^{-5}	8.67×10^{-6}	1.08×10^{-5}

Table 4: absorbance of the standard complex at 458nm with a fixed volume of KSCN.

Sr. No	Flask (25mL)	$Fe(NO_3)_3$ (mL)	KSCN (mL)	DW (mL)	Absorbance At 458nm (Abs)
1.	1	0	2.5	22.5	0.316
2.	2	5	2.5	17.5	0.615
3.	3	10	2.5	12.5	0.846
4.	4	15	2.5	7.5	1.027
5.	5	20	2.5	2.5	1.122

Table 5: absorbance of the sample at 458nm with a fixed volume of KSCN.

Sr. No	Sample code	Absorbance At 458nm (Abs)	Concentration (M)
1.	CM1	0.160	$2.53x10^{-6}$
2.	CM2	0.157	$2.48x10^{-6}$
3.	CM3	0.132	$2.08x10^{-6}$
4.	CK1	0.601	$9.50X10^{-6}$
5.	CK2	0.268	$4.24x10^{-6}$
6.	CK3	0.548	$8.67x10^{-6}$

Table 6: absorbance of Fe-OPT complex at 525nm.

Sr. No	Sample code	Absorbance At 525nm (Abs)	Concentration (M)
1.	CM1	0.099	1.13×10^{-5}
2.	CM2	0.070	7.30×10^{-6}
3.	CM3	0.087	9.10×10^{-6}
4.	CK1	0.278	2.84×10^{-5}
5.	CK2	0.148	1.55×10^{-5}
6.	CK3	0.187	1.95×10^{-5}

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