DETERMINATION OF THE HEAVY METALS AND PROXIMATE ANALYSIS FROM VEGETABLES IN HYDERABAD, SINDH

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ABSTRACT: Vegetables are the basic need for life and provide us macronutrients and micronutrients. Different food groups are consumed by human beings and each group has its own importance and provides nutrients in different proportions. Vegetables are considered one of the good sources of minerals and vitamins and widely consumed by humans in any age group and they are considered as best energy supplier, being these kinds of properties and importance, they are high in cost and demand. In this study trace metals from fourteen vegetable samples were analyzed such as Co, Cr, and Ni among the vegetables are ranging from (Cobalt = 1.027-1.46µg/g), (Chromium = 0.22-1µg/g), and (Nickel = 0.2-1.8µg/g). However most of the elements were present within the recommended range. Vegetable were also analyzed for their proximate composition such as moisture, carbohydrate, crude protein, crude fat, crude fiber, and ash contents.

Keywords: Heavy Metals, Nickle, Proximate, Vegetables.

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INTRODUCTION

Agriculture is a composite fact and exerts both unfavorable and favorable environmental consequences [1]. Agriculture is the main support of Pakistan's national economy. The second main concern is sector after defense. It shares about (24%) in GDP and contribute 35% in the earning of export and employs and 51% is of total labor force and play very important role in mobbing the life of 70% population of rural areas. Plenty of vegetables are grown in Pakistan and some of the vegetables such as Tomato and Chili are exported to other countries. Export share of vegetables is about 0.22% in Pakistan [2]. Environmental and soil pollution is worldwide a major problem and required great concern because it has adverse effects on health of humans, animals plants and exposed substances to Hg, Pb, As and Cd. Consumption of toxic heavy metals needs more attention worldwide due to carcinogenic, neurotoxin and many other impacts caused by the consumption of heavy metals through food chain [3]. Heavy metal are accumulated in food and causes several chronic effects in liver and kidneys of human beings and may disturbed many biochemical processes and causes nervous, kidney, cardiovascular and bone diseases [4]. The soil ecosystem is contaminated by heavy metals in all over the world due to human activities and uptake of metals through food chain causes risk to health of human [5]. Heavy metal contamination in land is day by day increasing and causing environmental and economical issues in Pakistan [6].Heavy metals are very toxic since they are water soluble. Smaller amount of metals can have detrimental effects on animals and humans as at this time no superior method is introduced for the exclusion of metals from the body. Metals are extremely using in industries and effluents from industries produces severe complications [7]. Heavy metals in waste water not only contaminated the soil but also the quality of food [8]. Heavy metal contamination through food chain has several hazards on health of human beings [9]. Heavy metals enters in to the vegetables as a result of natural and anthropogenic activities and uptake of heavy metals for a long period of time even at low concentration causes toxic effects on human health and make insure that contamination of heavy metals in food is below the maximum limits recommended by World Health Organization in mg/ml [10]. Continue ingestion of anxious uptake of heavy metals through agriculture which may leads prolonged build up of the metals in the liver and kidney of the humans and causing numerous biological procedures as nervous and cardiovascular diseases, bone infections and kidney failures [11]. Vegetable contains certain nutritional components due to this; consumption of vegetables is increasing day by day. Vegetables are essential for human health and are frequently known as defending food due to their different functions they prevent human health from various diseases. Regular intake of the investigated vegetables will supply the satisfactory amounts of the essential and trace metals required for humans, however will not comprise health hazard from toxic elements in order to prevent from a possible poisoning of heavy metals and as well as globally to ensure the safety of the public. Vegetables are considered as a defending complementary food. They have large quantity of Carbohydrates, Proteins, Dietary fibers, Minerals, Amino acids and vitamins which are necessary for normal functions of human metabolic process. Vegetables are also important to neutralize the acid which is produced during the process of digestion. In addition, according to the experts foods are being helpful "fiber" [12, 13, 14, 15, 16]. It has been reported that depositions of atmosphere considerably lift up the level of contamination of heavy metals in vegetables during marketing [17]. Vegetables are contaminated with heavy metals due to wastewater use for irrigation as well as use of fertilizers and pesticides, Urban, industrial emission and household activities, storage and methods of harvesting and transportation Significantly increasing the load of heavy metal pollution. Heavy metal accumulation in vegetables is due to contaminated soil and parts of the plants exposed to the air [18, 19]. Vegetables are enormous consequence resource of plant proteins, minerals and vitamins in human diet throughout the world. Cultivation of Vegetable are one of the most well-organized and major undergrowth of economic value and agriculture as well. The cultivation of vegetables is becoming very costly by purchasing fertilizers and pesticides to maintain the level of production.

MATERIAL AND METHODS

Samples Collection: Total 14 number of samples of fourteen varieties (one sample from each verities; comprising of 500g/sample) of vegetables such as Potato, Tomato, Cabbage, Cucumber, Ridged gourd, Apple gourd, Okra, Unripe Mango, Mint, Garlic, Spinach, Ginger, Lemon, and Onion) were purchased from different local markets of Hyderabad Sindh. Vegetables were transported to the analytical laboratory for pre-treatment and stored in a cool and dry place before the analysis.

Table 1: Vernacular (Urdu) and scientific names of vegetables studied.

S. No	Local name	English name	Botanical name
01	Aaloo	Potato	Solonumtuberosum
02	Tamater	Tomato	Solonumlycopersicm
03	Baandgobi	Cabbage	Brassica oleracea
04	Khera	Cucumber	Cucumissativus
05	Turai	Ridged gourd	loofa acutangula
06	Tenda	Apple gourd	Benincasafistulosa
07	Bhindi	Okra	Abelmoschus
08	Carry	Unripe Mango	Mangiferaindica
09	Podina	Mint	Menthalongifolia
10	Lahsan	Garlic	Allium Sativum
11	Palak	Spinach	Spinaciaoleracea
12	Adrak	Ginger	Zingiberofficinale
13	Nimbu	Lemon	Citrus Limon
14	Piaz	Onion	Allium cepa

Vegetable Size: The vegetable size was measured with help of Vernier's caliper and the average vegetable size (length and diameter) was calculated and expressed in centimeters (cm).

Vegetable Weight: Vegetables were selected at random and weighed on top pan balance individually was calculated and expressed in kg.

Determination of carbohydrates by different methods: The method involves in obtaining the available carbohydrate contents by calculation having estimated all the other fractions by proximate analysis, i.e.

Carbohydrate % = 100 – (% moisture + % Ash + % Fat +% Protein +% Fiber) [20]

Determination of crude fat: The Crude fat was determined by Mojonnier method

The following formula was used for the percentage fat content of the sample:

$$\text{Fat content \%} = \frac{W2 - W1}{W3} \times 100$$

Where, W1 = weight of empty flask (g), W2 = weight of flask + fat (g) and W3 = weight of sample taken (g).

Determination of crude protein: The micro Kjeldahl method [21] was used for the determination of crude protein.

Determination of crude fiber (%)

- Crude fiber was estimated by taking 2g sample and added with 1.25% H_2SO_4, then with 1.25% NaOH.
- The sample was filtered with Whattman paper No. 4.
- Washed with warm distilled water and then with 1% nitric acid and again with hot distilled water.

$$\text{Crude fiber } \% = \frac{(c-b)-(d-b)}{(a)} \times 100$$

Where a = weight of sample b = weight of crucible c = initial weight of crucible containing tissue sample before ignition and d = final weight of crucible containing ash after ignition.

Ash contents: Sample (5g) was transferred to a pre-weighed crucible and ignited into muffle furnace at 550^{o} C to obtain ash. The parentage ash was calculated as

$$\text{Ash contents} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Moisture Content: The moisture content was calculated using the AOAC [22] method. In pre-weighed dishes, ten gram of sample was dried in a hot air oven at 130 °C (±1 °C) until consistent weight. The dish containing the dried sample was placed in desiccators and allowed to cool to room temperature. After that, the dish was weighed, and the moisture content in percent was estimated using the weight loss.

Reagents and Chemicals: Stock solution of each metal for Vegetables was prepared by dissolving appropriate amount of individual salt in de-ionized water. Analytical grade Chromium Sulfate ($Cr_2(SO_4)_3$), Cobalt Sulfate ($CoSO_4$) and Nickel Sulfate ($NiSO_4$) were purchased from Merck (Darmstadt, Germany) and Hydrogen per oxide **(30%)** and Nitric acid **(65%)** were also purchased from Merck (Darmstadt, Germany). De -ionized water was used throughout the analysis.

Instrumentation: The analysis of the study was carried by using Perkin-Elmer Flame Atomic Absorption Spectrometer, with air–acetylene flame connected with an AA-800 auto-sampler and controlled by computer installed with Win Lab Software. The essential metals in all the digested sample solutions were analyzed on atomic absorption spectrometer and concentration of element in each sample was determined by calibration curve for each metal prepared using calibration standards. All the analysis was made in triplicate.

Sample pre-treatment of vegetables: Fresh samples of vegetables were first washed with tap water and then rinse with de-ionized water after washing samples were placed in Petri dishes; samples were air dried at room temperature. Next the edible parts of the vegetables were separated and after that oven dried at 70 ˚C until constant weight. The dried samples were cooled and grounded with the help of porcelain pestle mortar and stored in plastic begs for digestion Process.

Sample wet digestion of vegetables: Accurate 0.5gm dried powdered sample was taken and poured in to the cleaned and dry vessel and 5 ml conc. Nitric acid (HNO_3) was added and placed on the hot plate for 1 hour at 80 ˚C to become semi dried. After that conc. HNO_3 (5 ml) and H_2O_2 (2 ml) was added again followed by heating on hot plate for 1 hour at 80 ˚C. After getting semi dried the resulting sample was cooled and filtered with watt man filter paper in 25 ml volumetric flask and volume was made up with 2N HNO_3 up to the mark and taken to the Perkin-Elmer Flame Atomic Absorption Spectrophotometer for metal analysis.

Statistical Evaluation: The obtained data of the study were analyzed by using Microsoft Excel. One sample collected in triplicate and analyzed thrice and represented as Mean ± Standard Deviation.

RESULTS AND DISCUSSION

Proximate composition of each vegetable is shown in table 2. The Moisture content in all vegetable samples was very high ranging from 8.80% in Ginger and 92.33% in Ridged gourd. After moisture content the second major chemical constituent found was Carbohydrates with maximum range 84.21% in Onion and minimum content 1.6% in Cabbage. The crude fiber content with maximum concentrations 74.2% was determined in Ginger, while minimum amount 0.15% was found in Tomato. Crude fat content observed was 0.03% in Onion and 8.72% in Garlic. Crude protein was estimated 0.63% in unripe mango and 26.8% in Tomato, while Spinach showed second high concentration 23.48%. Ash contents were 0.21% in unripe mango and 21.87% in Spinach. Vegetables are very rich sources of essential biochemical and nutrients such as Carbohydrates, Vitamins, Iron and Calcium, Carotene, Ascorbic acid and palpable concentrations of trace minerals [23].

Table 3 shows the results of trace metals uptake in vegetable plants absorbed from the soil of the cultivated area, the atmospheric condition and partly from the irrigated water. In this study trace minerals such as Co, Cr and Ni were done by atomic absorption spectroscopy in µg/g. In present study the lowest Cobalt content was found 1.027 µg/g in Potato and highest was observed in Cucumber 1.46 µg/g. The daily recommended range of Co in human diet is 0.005 mg/day [24]. The lowest Chromium contents was found 0.22 µg/g in Apple gourd and highest was found in Onion as 1 µg/g. Chromium is present in variable concentrations in tissues of human body and its deficiency is characterized by disturbance in lipids, proteins and glucose metabolism [25]. Nickel was found higher such as 1.8 µg /g in onion and lowest were observed in Potato and Spinach such as 0.2 µg/g. The acceptable range of Ni daily intake is 3-7 mg/day. The study concluded that most of the elements were present within the recommended rang.

Table 2: Proximate composition (%) of different vegetables.

Vegetables	Moisture	Carbohydrates	Crude Fiber	Fat	Crude Protein	Ash
Potato	74.83±1.3F	22.8±0.3H	1.8±0.07G	0.2±0.01I	1.8±0.03I	1.3±0.03K
Tomato	48.13±1.2H	6.55±0.2K	0.15±0.01H	1.56±0.08G	26.8±0.3A	16.12±0.2B
Cabbage	90.81±1.3AB	1.6±0.04L	2.6±0.03F	0.21±0.01I	2.1±0.08I	1.9±0.09I
Cucumber	92.32±1.5A	63.52±0.7D	7.20±0.09E	2.63±0.03E	9.56±0.1G	9.5±0.1E
Ridged gourd	92.33±1.4A	70.63±0.8B	11.54±0.2C	2.96±0.02D	11.47±0.2F	5.7±0.1G
Apple gourd	90.33±1.2B	37.95±0.8G	8.47±0.09D	2.63±0.09E	14.33±0.2E	11.65±0.2C
Okra	84.62±1.1D	64.02±0.7D	15.36±0.3B	6.13±0.07B	15.21±0.3D	9.26±0.1E
Unripe Mango	23.36±0.4I	20.03±0.7I	7.03±0.09E	0.21±0.01I	0.63±0.01J	0.21±0.01L
Mint	80.21±0.9E	54.21±0.9F	7.02±0.09E	2.51±0.06F	6.58±0.09H	10.82±0.3D
Garlic	72.68±0.9G	59.27±0.9E	1.75±0.06G	8.72±0.09A	20.50±0.3C	3.82±0.09H
Spinach	91.52±1.3AB	38.46±0.9G	6.82±0.08E	2.57±0.07EF	23.48±0.3B	21.87±0.3A
Ginger	8.80±0.09J	68.26±1.3C	74.2±0.8A	4.02±0.09C	6.77±0.09H	1.62±0.02J
Lemon	84.12±1.1D	11.51±0.4J	1.63±0.05G	1.17±0.01H	11.43±0.2F	0.40±0.01L
Onion	87.51±1.2C	84.21±1.4A	0.21±0.01H	0.03±0.01J	0.89±0.01J	6.1±0.09F
F-Statistics	1712	3318	18267	5556	5896	5537
P-Value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

(Values are expressed as Mean ±Standard deviation (n=3); Mean values followed by the same letter down the column were not significantly).

Table 3: concentrations of trace metals in vegetables.

S.N0.	Vegetables	Cobalt (Co) µg/g	Chromium (Cr) µg/g	Nickel (Ni) µg/g
01	Potato	1.027±0.02I	0.5±0.01GH	0.2±0.007K
02	Tomato	1.13±0.02F	0.51±0.01FG	0.4±00.01J
03	Cabbage	1.41±0.02C	0.46±0.01I	0.9±0.02G
04	Cucumber	1.46±0.02B	0.6±0.01D	1.5±0.05C
05	Ridged gourd	1.62±0.04A	0.57±0.01E	1.2±0.04D
06	Apple gourd	1.41±0.02C	0.22±0.01K	0.5±0.01I
07	Okra	1.1±0.02FG	0.69±0.01C	0.7±0.01H
08	Unripe Mango	1.25±0.02D	0.7±0.01C	1.1±0.03E
09	Mint	1.45±0.02BC	0.91±0.02B	0.5±0.03I
10	Garlic	1.19±0.03E	0.4±0.01J	1±0.02F
11	Spinach	1.189±0.04E	0.92±0.02B	0.2±0.01K
12	Ginger	1.24±0.03D	0.52±0.01F	1.1±0.04E
13	Lemon	1.076±0.02GH	0.49±0.01H	1.6±0.06B
14	Onion	1.04±0.02HI	1±0.01A	1.8±0.07A
	F-Statistics	158	998	642
	P-Value	0.0000	0.0000	0.0000

(Values are expressed as Mean ±Standard deviation (n=3); Mean values followed by the same letter down the column were not significantly).

Conclusion: In present study, it was found that vegetables contain sufficient amounts of essential elements which are important for the proper development of the body. In addition, the concentrations of potentially trace elements were usually found below to the limits suggested by WHO. This study suggested that fresh vegetables should be included in daily diet because vegetables are being a safe and good source of essential and trace elements than other sources of supplementations. It was observed that in all the selected vegetables the compositions of nutrients were different. The maximum content of carbohydrate was observed in ridged gourd, and highest protein contents were observed in tomato. It is concluded from this research that vegetables are nutritious foods so as to supply adequate quantity of nutrients which are required for normal functions of the body, reproduction and maintenance. Heavy metals have a toxic impact on human beings, although a harmful impact appears after the long term consumption of heavy metals contaminated vegetables. This study has a great importance because human health is directly affected by ingestion of vegetables consequently the biomonitoring of trace elements in vegetables, desires to be continued for the reason that these are the major food sources for humans in various parts of the world.

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