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Comparative nutritional analysis of *Spinacia oleracea* in different cities of west uttar pradesh (INDIA)

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ABSTRACT

Green leafy vegetables are the rich sources of essential minerals and vitamins. The natural compositions of such vegetables have huge importance in the field of cosmetology and treatment of various diseases. In the present work, *Spinacia oleracea,* belonging to *Chenopodiaceae* family, were analyzed for basic nutrients in the diet with a view to ascertain its nutritional potentials variation according to geographical area. The study found that nutritional potential of the spinach cultivars namely Agra, Mathura and Aligarh had moisture content of 94.3% on dry basis. Phytochemical screening of the *Spinacia oleracea* showed the presence of various vitamins, minerals and amino acids. The study revealed that these spinach cultivars possess nutritional and health benefits for diabetic patients, individual that are watching their weight and those with ischemic heart disease.

Keywords: Nutritional potential, Phytochemical, *S. oleracea,* Nutraceutical.

1. INTRODUCTION

Spinacia oleracea, commonly known as Spinach (English), Chhurika (Sanskrit), Palak (Hindi; Gujarati; and Marathi), Palakh (Kashmiri), Palang (Bangla), Pasalai (Tamil), and Mathubucchali (Telugu)[1], is an extremely nutritious leafy vegetable, rich both in core nutrients and phytochemicals[2]. In different traditional medicinal system it is known by different names. It's Ayurvedic name is 'Paalankikaa', in 'Unani' it is called as 'Paalak', where as in 'Siddha' it is known by 'Vasaiyila-keerai'[3]. *Spinacia oleracea* Linn.(SO) is an annual plant having medicinal property native to central and south western Asia. It is cultivated for the sake of its succulent leaves. It has the largest consumption as favourite food in winter season of India [4].

Spinach is a mineral-rich vegetable. An earlier study on the edible portion (87%) of spinach records (in %): moisture, 94.3; protein, 2.2; fat,0.7; fiber,0.6; mineral matter,1.7; carbohydrate, 2.9; and oxalic acid, 658 (mg/100g). Mineral composition includes (mg/100g): calcium,73; magnesium, 84; potassium, 206; iron, 10.9; phosphorus, 21; sodium, 58.5; copper, 0.01; sulphur, 30; nickel, 0.42; manganese, 9.61; molybdenum, 0.08; zinc, 13.53; and strontium, 0.077. Spinach is a good source of the vitamin B complex, ascorbic acid, vitamin A and carotene. It is also a natural source of vitamin K [5].

In the present paper, an extensive study on the mineral elements, vitamins and amino acid compositions of spinach leaves and stems from various locations of Uttar Pradesh (India) has been reported. Twenty two local spinach samples, collected at different times and from different places, were analyzed for 6 elements, vitamins and amino acid using Perkin–Elmer A- Analyst 800 atomic absorption spectrometer by suitable hollow cathode lamps after the digestion. The objective of the present study was to provide a comprehensive account of the mineral elements, vitamins and amino acid values in spinach and find out their pattern of occurrence in spinach leaves and stems.

2. Material and Methods

2.1. Reagents and samples

All the solvents (Analytical Grade) were purchased from Rankem (India). HNO_3 and $HClO_4$ were also purchased from Rankem (India). Analytical grade Riboflavin, thiamine and L-ascorbic acid and amino acids standard were

purchased from Himedia (India). *Spinacia oleracea* leaves were collected from three different cities of west Uttar Pradesh in India. Specimens are preserved in the institute herbarium of K.R. Collage Mathura, Uttar Pradesh, India. *Spinacia oleracea leaves* were thoroughly washed with water and dried in air oven at 40° C for 72 h for further use. For HPLC analysis, millipore water was used throughout the studies. The stock and standard solution were prepared in mobile phases.

Moisture content of *Spinach-oleracea* leaf was determined according to an air-oven method. Ash content was determined by incinerating at 410-440 ^{0}C until the constant weight was achieved.

2.2. Instrumentations

Mineral nutrients in *Spinacia oleracea* leaves were analyzed using a Perkin–Elmer A-Analyst 800 atomic absorption spectrometer by suitable hollow cathode lamp after the digestion of ash of leaves using HNO_3, H_2SO_4 and $HClO_4$ acid and diluting with double distilled water to a specific volume.

Vitamins (riboflavin, thiamine and ascorbic acid) and amino acids were analyzed using reverse phase high performance liquid chromatography using waters HPLC system. The HPLC system consists of water 1525 binary HPLC pump and 717 plus auto sampler (waters®). The system was operated at ambient temperature. The chromatographic peaks of amino acids were identified and quantified by Breeze™ software (Version 3.2). Amino acids were analyzed AccQ Tag™ reverse phase (3.9×150 mm) 4 µm analytical column equipped with 2475 multi fluorescence detector (emission and excitation wavelength 395 and 250nm). Cystine and Methionine were analyzed from the same method of acid hydrolysis after treatment using performic acid oxidation while vitamins (riboflavin, thiamine and ascorbic acid) were analyzed using an octadecyl end capped RP-C18 column (4.6 mm i.d. ×25 cm) 5 µm pore size equipped with a UV detector.

2.3. Preparation of standard solution

Standard solution of ascorbic acid was prepared by dissolving 50 mg of ascorbic acid in meta-phosphoric acid (0.3 M) and acetic acid (1.4 M) solution (1:4 ratio) at the final concentration 1mg/ml. Standard solution of riboflavin was prepared by dissolving 50 mg riboflavin in double distilled water followed by addition of three to four drops of glacial acetic acid and warming the solution to 85°C. Final concentration of the riboflavin was made to 100 µg/ml where as the standard solution of thiamine was prepared by dissolving 26.7 mg of thiamine hydrochloride in 25 ml of doubly distilled water.

2.4. Chromatographic conditions

Many analytical methods have been reported by various researchers for the determination of thiamine, riboflavin and ascorbic acid [6-8]. Selection of method generally depends upon accuracy, sensitivity and the interferences encountered in the sample matrix. Chosen method for determination of thiamine, riboflavin, and ascorbic acid were identified by comparing the retention time of the sample peak with that of the thiamine, riboflavin, and ascorbic acid standard at 250, 270 and 254 nm. Quantification was carried out using external standard. For the identification of thiamine, riboflavin mobile phase (12.5 mM sodium acetate in a mixture of methanol/ water 25/75 mM sodium heptane sulphonate) with a flow rate of 1.0 ml/min was used while for the identification of ascorbic acid mobile phase (0.1M potassium acetate pH 4.9 in a mixture of acetonitrile water 50/50) with a flow rate of 1.4 ml/min was used.

2.5. Sample preparation for analysis of trace elements

A 50.0 g of *Spinacia oleracea* leaves were crushed, grinded in a mortar. Dry ashing method was adopted by placing the properly dried sample into the versatile crucible overnight in an electric muffle furnace maintaining the temperature between 400-440 °C. This ashing will destroy all the organic material from the sample. The ash was removed from crucible and dried in desiccators. The yield of ash was appox. 6.5 g/ 100g. A 1 gm of ash was taken and digested using conc. HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 10:6:3. Digested ash was stored in sterilized bottles and used for the determination of Ca, Zn, Mg, Fe, Na, and P by flame atomic absorption spectroscopy. Phosphorus was analyzed with colorimeter using ammonium vanadate-molybdate method. Three replicates were prepared for each sample.

2.6. Sample preparation for analysis of vitamins

Riboflavin and thiamine were extracted using the method described in literature [9]. One gram of *Spinacia oleracea* leaves powder was transferred into a 50 ml graduated polypropylene centrifuge tube and followed by the addition of 20.0 ml of 0.1 H_2SO_4. The mixture was shaken vigorously for 1 min, and then placed in boiling water for 30 min and shaken at 5 min intervals. Now the mixture was cooled in an ice bath and followed by the addition of 2.5 ml of 2% a-amylase. After mixing properly, the mixture was

incubated at 50°C for 1 hr in a water bath with shaking. The mixture was cooled and then diluted to 25 ml with deionised water. The resulting mixture was centrifuged. The supernatant was filtered through a 0.45 µm nylon filter disc before HPLC analysis. All samples were carried out in triplicate.

Vitamin C was extracted using the modified method of Abdulnabi et al.[10]. One gram of *Spinacia oleracea* leaves powder was homogenized with an extracting solution containing meta-phosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask (wrapped with aluminum foil) and agitated at 100 rpm with the aid of an orbital shaker for 15 min at room temperature. Mixture was then filtered through a Whatman filter paper No. 4 to obtain the clear extract. The sample to extraction solution ratio was 1:1. All samples were extracted in triplets.

2.7. Sample preparation for analysis of amino acids

Total nitrogen and the protein content were determined based on the Kjeldahl method using the conversion factor of 6.25. All the above determination were based on the method of AOAC (1990) [9].

The sample was hydrolyzed in triplet using 6N HCl at 110 ^{o}C for 24 h and derivatized using AccQ reagent (6'Aminoquinol-N-hydroxysuccinimdyl carbamite) [11].

3. RESULTS AND DISCUSSION

3.1. Minerals

In the present research the trace minerals such as Ca, Zn, Mg, Fe and Na were done by using atomic absorption spectroscopy in mg/100g. The moisture contain in *Spinacia oleracea* leaves is 94.6%

Trace metals in fruit and vegetable are plants absorbed from the soil of the cultivated area, the atmospheric condition and partly from the irrigated water. Iron content in *Spinacia oleracea* leaves ranged from 60 mg/100g to 90 mg/100g. Maximum iron content was found at site-c Agra and the minimum was noted at site-c Mathura. Iron content analyzed in the present study was similar to that reported by Yadav et.al [12] in spinach (35.0 mg/100 g) and lower than that reported by Luthra & Sadana[13] in the same vegetable (54.1 mg/100 g). Ionizable iron, as a percent of total iron in spinach, was found quite similar to the findings of other workers [14, 15], who reported 4.4 and 5.0 percent of total iron in spinach. Various factors that affect iron content of leaves are stage of maturity, conditions of growth, fertilizers used and nature of soil [16]. Iron is one of the essential metal needed in various enzymatic reactions and it's daily requirement is ranged from 1.5-2.2 mg/day [17]. Zinc content in *Spinacia oleracea* leaves ranged from 13 mg/100g to 27 mg/100g. Maximum zinc content was found at site-a, Agra and the minimum was noted at site-c, Mathura. According to the WHO recommendation, fruits and vegetables are poor sources of Zn and ranged upto 1 mg/kg and dietary intake for Zn is 14-20 mg/day [18]. Some workers reported 7.0mg/100g zinc in spinach [19] Sodium content in *Spinacia oleracea* leaves ranged from 193 mg/100g to 310mg/100g. Maximum sodium content was found at site-c, Aligarh and the minimum was noted at site-b, Mathura. Na as an essential macro element has physiological effect in human and animal cellular and metabolic mechanism. The increased level of Na content has direct link to the high blood pressure [20]. The daily recommended range of Na in developing countries is between 2400-5175 mg/day [21]. Calcium uptake in spinach was higher *i.e* 310mg/100g at site-b, Aligarh than 211 mg/100g at site-c, Agra. It controls the membrane structure, membrane permeability and provides the stability to cell [22]. Calcium is essential for healthy bones, teeth and blood[23]. The health of the muscles and nerves depends on calcium. The recommended daily allowance of Ca for children is between 500mg and 1000 mg and for adults 800 mg[24]. Magnesium maximum uptake was found in the range 215mg/100g to 264 mg/ 100g. Magnesium daily dietary intake ranged from 400 to 420 mg/day [25]. The concentration of phosphorus was found in the range between 217 mg/100g to 264 mg/gm. The high phosphorus concentration was found at site-c, Agra while site-c, Mathura showed low phosphorus concentration. The balance of phosphorus and calcium is regulated by parathyroid hormone, which increases urinary excretion of phosphate under conditions of high phosphate and low calcium intake [26]. Recommended dietary allowances have been set at 460–1250 mg of phosphorus per day for different age groups by the United States Institute of Medicine [27]. The values are reported in Table-1 and the relative graphical representation of these vitamins according to their sampling sites is shown in Figure-1.

3.2. Vitamins

The comparative study was conducted to determine the presence of different vitamins viz. Thiamine, Riboflavin and Ascorbic acid (vitamin C) in *Spinacia oleracea* leaves, collected from different sample sites. The concentration of Thiamine, Riboflavin and Ascorbic acid reported in Table-2. Ascorbic acid content in *Spinacia oleracea* leaves of different sites varied from

Table -1: Concentration of trace elements (mg/100g) in leaves of Spinach at different sampling sites

Spinach (mg/100g)	Total Ash: 6.5 g/ 100g								
	Site-I (Aligarh)			Site-II(Mathura)			Site-III (Agra)		
Element	a	B	C	a	b	c	a	b	c
Na	237	256	310	215	193	220	263	252	285
Mg	237	215	247	233	226	217	215	241	264
P	147	114	135	165	142	122	188	175	165
Ca	290	310	316	215	281	245	265	245	211
Fe	80	86	69	76	62	60	66	85	89
Zn	6.6	6.2	6.2	5.9	5.2	5.1	6.8	6.2	6.6

Table - 2: Concentration of vitamins (mg/100g) in leaves of Spinach at different sampling sites

Spinach (mg/100g)									
Vitamins	Site-I (Aligarh)			Site-I(Mathura)			Site-III (Agra)		
	a	b	C	a	b	c	a	b	c
Thiamine	0.15	0.17	0.15	0.16	0.15	0.16	0.16	0.17	0.16
Riboflavin	0.47	0.45	0.50	0.53	0.49	0.50	0.54	0.51	0.54
Vitamin C	36.8	36.5	35.0	36.5	36.8	36.9	37.1	36.9	36.7

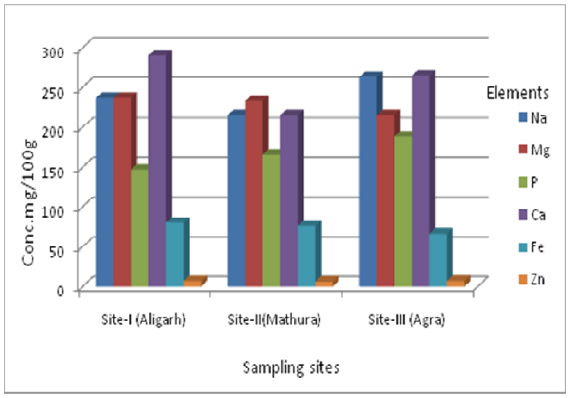

Figure - 1: Trace Elements in Spinach leave at different sampling sites.

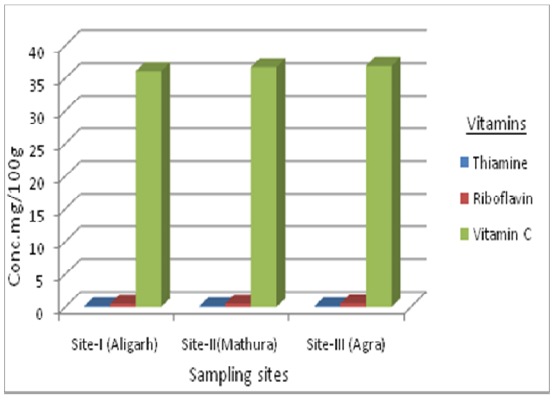

Figure - 2: Vitamins in leaves of Spinach at different sampling sites.

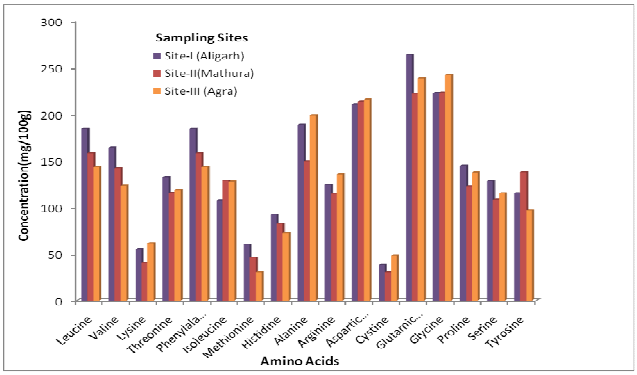

Figure - 3: Concentration of Amino Acids (mg/100g) in leaves of Spinach at different sampling sites.

Table - 3: Concentration of Amino Acids (mg/100g) in leaves of Spinach at different sampling sites.

Spinach (mg/100g)									
	Site-I (Aligarh)			Site-II(Mathura)			Site-III (Agra)		
Essential amino Acids	**a**	**b**	**C**	**a**	**b**	**c**	**a**	**b**	**c**
Leucine	189	179	185	165	152	158	148	139	143
Valine	165	158	168	144	137	144	128	122	119
Lysine	55	58	52	42	38	41	62	58	64
Threonine	136	132	128	112	118	116	114	119	122
Phenylalanine	189	179	185	165	152	158	148	139	143
Isoleucine	102	108	112	132	128	125	125	131	126
Methionine	59	55	65	44	46	48	32	31	28
Histidine	92	94	90	87	82	78	76	72	69
Non essential amino acids									
Alanine	195	178	191	145	155	148	198	192	205
Arginine	122	124	126	119	112	113	138	132	136
Aspartic acid	203	216	212	210	218	212	215	212	219
Cystine	35	38	42	30	33	28	44	48	52
Glutamic acid	269	262	259	225	219	221	235	239	241
Glycine	225	220	222	216	228	224	242	238	246
Proline	143	149	141	118	123	126	132	138	141
Serine	124	132	128	104	108	112	111	118	116
Tyrosine	112	115	118	132	138	142	96	92	102

35.0 mg/100g to 37.1 mg/100g. A significant variation was observed in Ascorbic acid content in *Spinacia oleracea* leaves. Almost similar values have been reported earlier [28].

Various regions including differences in soil analysis, different environmental conditions and maturity of plant [29]. Ascorbic acid act as antioxidants and potentially act as anticancer agent because Ascorbic acid acts in the stomach as scavenger of nitrites and free radical formed during metabolic processes [30].

The concentration of Thiamine (vitamin B_1) and Riboflavin (vitamin B_2) were found in the range between *0.15* mg/100g *to 0.17* mg/100g and 0.20 mg/100g *to 0.24* mg/100g at different sampling sites respectively. The relative graphical representation of these vitamins according to their sampling sites is shown in Figure-2. Recommended dietary allowance for Riboflavin for adult is 1.3 mg per day form man and 1.1 mg per day for woman [31]. Thiamine intake through supplement is 2.4 mg per day for man and 3.2 mg per day for woman. Approximately 27% of adults took thiamin supplements to recover deficiency of Thiamine [32].

3.3. Amino Acids

The investigated material was uniform, and has been collected from different sampling sites of west Uttar Pradesh, in order to estimate the effect of various factors on the chemical composition of the raw material. The protein content in *S.oleracea* is 2.46 gm/100gm. Essential amino acids constituted 38.96% of total amino acids as reported in Table-3. Glutamine and asparagines was expressed as glutamic acid and aspartic acid respectively.

On analyses of obtained results it was found that glutamic acid was found highest average values 241mg/100g followed by glycine 229mg/100g, aspartic acid 213mg/100g, phenylalanine and leucine162 mg/100g and valine 142mg/100g.The average concentration of glutamic acid was found highest at site –I(a), Aligarh and lowest at site II(a)Mathura. The concentration of glycine was found highest at site-I (a) Aligarh and lowest at site II(c) Mathura. Similarly, the concentration of Aspartic acid, Leucine are found highest value at site-III(c) Agra and Valine at site-I (a) Aligarh, while lowest concentration of these amino acids were found at site-I(a) Aligarh, site II(b) Mathura, site III(b) Agra and site III(c) Agra respectively.

Cystine, Methionine and Histidine were found lowest concentration among all the amino acids present in *S.oleracea*. The average value Cystine was found lowest at site-II, Mathura and high at site-III, Agra respectively. Similarly, Methionine and Histidine were found minimum at site-III, Agra. Whereas the higher values of these amino acids were found at site-I, Aligarh.

Similar trends in different amino acids has also been reported by Eppendorfer and Bille[33]. According to Eppendorfer [34] and Eppendorfer and Bille [33], spinach leaves contained 84–91 gm amino acids in 16 g N, depending on nitrogen fertilization, while the proportion of essential amino acids was 40–49%.

In the investigated material, the dominant amino acids were found glutamic acid and aspartic acid. In the amino acid composition of various vegetable species reported by several researches glutamic acid and aspartic acid were also found dominated [35,36]. The concentration trends in different amino acids, glumatic acid was found highest values whereas cystine, methionine and histidine were found lowest values [37].

4. CONCLUSION

The spinach cultivars are nutritious food that provide sufficient amount of nutrients needed for normal body function, maintenance and reproduction. Results concluded from the study indicate that the spinach can serve as a good nutritional source in combating malnutrition. The presence of various trace metals, vitamins and essential amino acids is an affirmation of the use of this leafy vegetable in the management of various ailments, and thus may serve as a source of ingredient to the pharmaceutical industries. The results obtained serves as a nutritional data base for local consumers, as well as for further research purposes.

Combination with other food stuffs is recommended to satisfactorily meet nutritional needs. Further research needs to be carried out on domesticating the under-utilized vegetable as it is often harvested from the wild, as well as carrying out animal trials to authenticate its tradomedical uses by rural communities. Spinach cultivars are poor source of fat that make them good food for obese and diabetic people.

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