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NUTRITIONAL ANALYSIS USING XRF TECHNIQUE AND CHLOROPHYLL ESTIMATION OF TWO VARIETIES OF LETTUCE

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ABSTRACT

Lettuce has been designated as "queen of salad plants" in the leafy vegetables plant groups. Two varieties of lettuce (*Lactuca sativa* Linn.) were taken in this study to estimate their chlorophyll and mineral contents. Lettuce can be cooked or eaten fresh as a salad. The nutritional status varies with the varieties and generally it is rich in vitamin K and A. Pigments like chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were estimated by the help of spectrophotometer. It was found that in *L. sativa* red leaf varieties, higher amount of chl a, chl b and total chlorophyll with a value of 23.920mg/ml, 43. 207 and 67.126 mg/ml respectively than green leaf with 21.795, 39.759 and 61.552 mg/ml respectively. Carotenoid content was maximum of 260.41 mg/ml in green leaf lettuce variety than in red with 246.01 mg/ml. Among all the mineral elements, high amount of potassium was estimated in red varieties with 1.685%, while green varieties had 0.917% of potassium. About 95.914% and 96.83% of water was found in red and green varieties respectively. High water content also is helpful for increasing activity of these leafy vegetables.

Keywords: Chlorophyll, carotenoid, *Lactuca sativa* Linn, Lettuce, mineral elements

INTRODUCTION

Vegetables are edible parts or whole plants and are consumed raw or cooked. They are consumed wholly or in parts, raw or cooked as part of main dish or salad. Leaves, stems, roots, rhizomes, bulbs, tubers, flowers, fruits and seeds are used as vegetables. (Uzo, 1989; Uwaegbute, 1989). Green leafy vegetables play an important role in human nutrition. About 48 green plant species are reported to be used as leafy vegetables by the seven tribes viz. Bathudi, Binjhal, Gond, Oraon, Sabara, Saura and Santal belonging to 8 species from Amaranthaceae, 5 from Fabaceae, 4 species both from Brassicaceae and Cucurbitaceae and 3 leafy greens from Caesalpiniaceae (Sagarika, P., Gyanranjan M., 2020). They provide sufficient amount of dietary fibers, minerals, vitamins and other nutrients to the people in the developing countries. Apart from the variety which they add to the menu (Asaolu *et al.,* 2012**),** they are valuable sources of nutrients especially in rural areas where they contributes substantially to minerals, vitamins, fibres, proteins, and other nutrients which are usually in short supply in daily diets. Leafy vegetables are the protective foods and prevent from various diseases as they contain minerals and vitamins (Mohammed and Sharif, 2011). It is also recommended for weight management because if low energy content (Nwanekezie and Obiakor, 2014). Leafy vegetables carry many minerals like Fe, Ca, P, Cu, Cl, Zn and Na. They have alkalizing effect against acidity produced by other foods (Angela *et al*., 2010). Vitamins are required for maintaining human health. Vitamin C is an important water-soluble vitamin and plays a major role in synthesizing collagens and carnitine. This vitamin is known to treat cold and secondary bacterial or viral infection (Rahman *et al*., 2006). Data revealed that sufficient consumption of vitamin C reduces the risk of developing cancers in breast, cervix, rectum, colon, lungs, prostate, mouth and stomach. Chlorophyll is an antioxidant and is also important to lower the blood sugar, indigestion, detoxification of liver and kidney and in lowering allergens and protecting the gastric mucosa (Kimura *et al*., 2007**).** It helps in boosting the immune system. Eight different leafy vegetables in south eastern Nigeria revealed presence of high quantity of Ca, Mg, Na and K with 63.36 to 110.16, 27.51 to 288.65, 15.01 to 88.00 and 16.85 to 168.96 mg/100g respectively. Low levels of copper (nd-3.14), nickel (2.32-18.16) and manganese (2.54-10.06) mg/100g were detected (Asaolu *et al.*, 2012). It was reported that vitamin A, vitamin C, vitamin E was found to be highest in *Moringa oleifera* (108.48 mg/ 100ml) followed by *Solanum nigrum* (3.18mg/100ml), *Cridoscolus acontifolius* (7.71mg/100 ml) and lowest in *Ficus capensis* (25.22mg /100ml), *Mucuna pruriens* (0.08 mg/100ml), *F. capensis* (3.39 mg/100ml). In this experiment vitamins of leafy vegetables were determined by the described official methods of the Association of Official Analytical Chemists (AOAC, 1990) and atomic absorption spectrophotometer was used to analyze the minerals contents. Nutritional value was estimated from 9 herbs and only one tree species based on the chlorophyll content of leaves and reported high content of chlorophyll a (1.902 mg /g), chlorophyll b (0.802 mg/g), total chlorophyll (2.703 mg /g) found in *C. halicacabum*, high content of chlorophyll a (0.898 mg/g), chlorophyll b (0.337 mg/g), total chlorophyll (1.235 mg /g) found in tree species *Barringtonia acutangula* (Vivek *et al.,* 2013). The micro and micronutrients of microgreens of *Trigonella-foenicum-graecum* L., *Brassica juncea* L and *Coriandrum sativum* L., were reported to contain high nutritional value in comparison with mature greens

and also the nutrient content reported to be varied with the growing growth medium and time of harvesting (Sagarika, P., 2020).

Lactuca sativa Linn. is commonly known as lettuce amongst the leafy vegetables. It is an annual plant and easy to cultivate. It requires low temperature for the growth. It is a laticiferous herb that belongs to Asteraceae family. Lettuce is added to sandwiches for extra flavor and colour. As per the interest of consumer it can be cooked or eaten fresh as a salad. The nutritional qualities according to the varieties and it contains good amount of vitamin K and A. It has mild flavor. Two varieties viz. red leaf and green leafy lettuce were taken in this study.

MATERIALS AND METHODS

Collection of Plant Samples

Two varieties of *Lactuca sativa* L. viz. green leaf lettuce and red leaf lettuce were selected from the kitchen garden of Centurion University of Technology and Management, Bhubaneswar, to quantify the chlorophyll and mineral element composition (Fig. 1 & Fig. 2). Healthy and uninfected lettuce variety was collected at their stage of maturity. During sampling of lettuce leaves care was taken

Table 1. Absorbency values of leaves of two varieties of *L. sativa* in different wavelength

Parameters	***L. sativa* (Green)**			***L. sativa* (Red)**		
Wavelength	663.2 nm	646.8 nm	470 nm	663.2 nm	646.8 nm	470 nm
Absorbency values	2.326	2.401	2.831	2.548	2.614	2.712

Table 2. Estimation of pigments in two varieties of *L. sativa*

Pigments	***L. sativa* (Green)**	***L. sativa* (Red)**
Chlorophyll a (mg/ml)	21.795	23.920
Chlorophyll b (mg/ml)	39.759	43.207
Total chlorophyll (mg/ml)	61.552	67.126
Carotenoids (mg/ml)	260.41	246.01

Table 3. Pigment contents in response with the days of the seedlings

Days of plants	**Total chlorophyll content**	
	Green leaf lettuce	**Red leaf lettuce**
10-30	Increasing	Increasing
31-60	Increasing	Increasing
61-90	Stable/maximum	Stable
>90	Decreasing	Decreasing

Table 4. Mineral contents in red and green lettuce

Lettuce			
Red Leaf Lettuce (*Lactuca sativa* L.)		**Green Leaf Lettuce (*Lactuca sativa* L.)**	
Elements	**Quantity (%)**	**Compounds/Elements**	**Quantity (%)**
SiO_2	0.519	SiO_2	0.742
P_2O_5	0.618	P_2O_5	0.562
SO3	0.210	SO3	0.135
Cl	0.367	Cl	0.384
K_2O	1.685	K_2O	0.917
CaO	0.650	CaO	0.384
MnO	0.00405	MnO	0.00283
Fe_2O_3	0.01745	Fe_2O_3	0.01441
ZnO	0.00271	ZnO	0.00163
Eu_2O_3	0.00454	Eu_2O_3	0.00411
Rb_2O	0.00182	Rb_2O	0.00091
SnO_2	0.00622	SnO_2	0.00566
Re	0.00015	Re	0.00012
H_2O	95.91	H_2O	96.83
SrO	0.00049	Er_2O_3	94.7
CuO	0.00092		

Fig. 1 - *Lactuca sativa* (green leaf lettuce)

Fig. 2 - *Lactuca sativa* (red leaf lettuce)

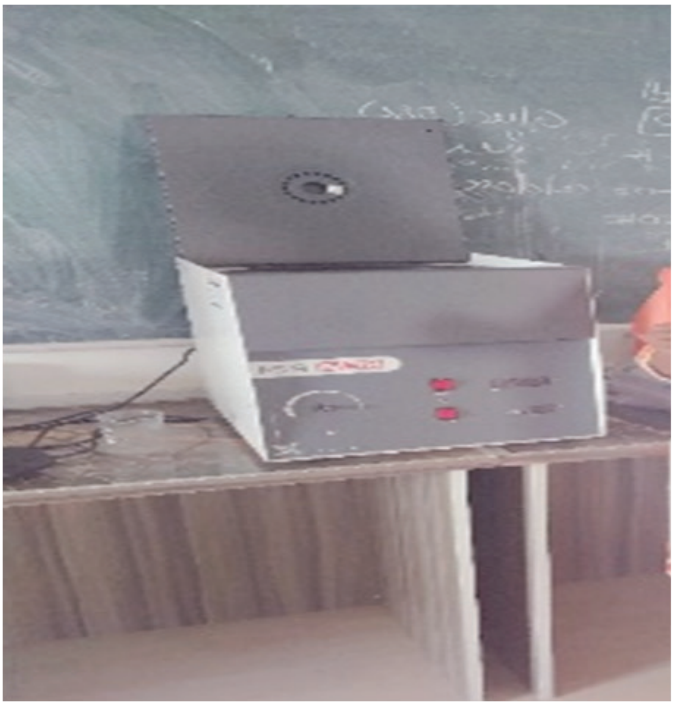

Fig. 3 - Spectrophotometer

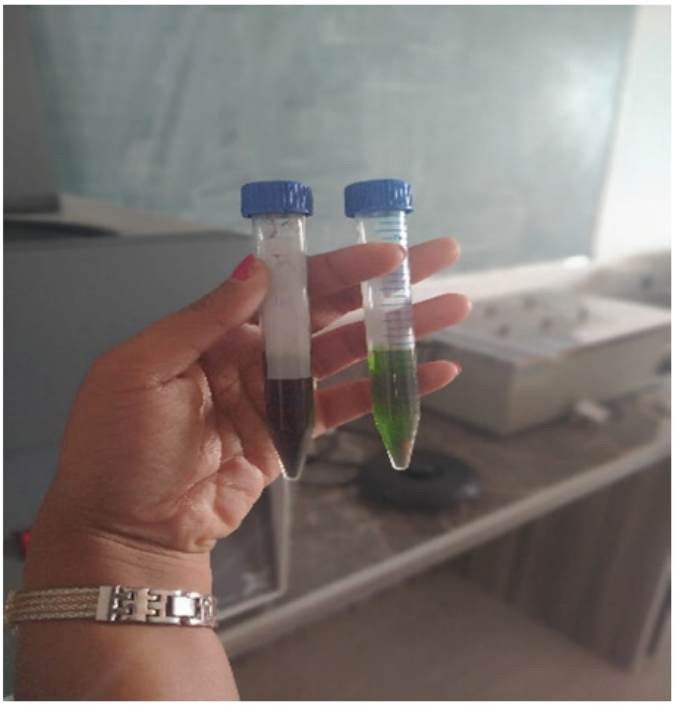

Fig. 4 - Samples for estimation of pigments

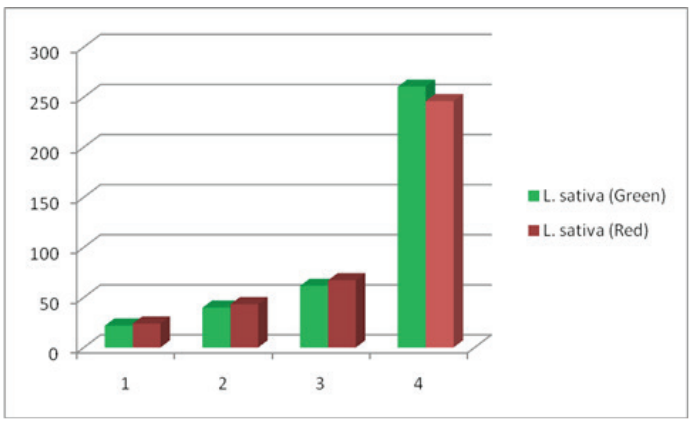

Fig. 5 - Pigments present in two lettuce variety

to avoid mechanical injuries. In the laboratory , first Fresh leaf samples were wash thoroughly in tap water followed by distilled water , kept to dry in room temperature (18°C) and analyzed for the determination of chlorophylls (Ch-a and Ch-b) and carotenoids content .

Chlorophyll Analysis

Accurately 1 gm of weighted fresh leaves were taken and ground with 10 ml of 80% acetone. Then it was centrifuged at 4120 rpm for 14 minutes .The supernatant was separated. 0.4ml of it is mixed with 4.4ml of the acetone. The solution was analyzed for the presence of Chl a, Chl b and carotenoid by the help of spectrophotometer (Fig. 3 & 4). Calculation was done to estimate the concentrations (mg/ml) of Chl a, Chl b and total chlorophyll (a+b) and

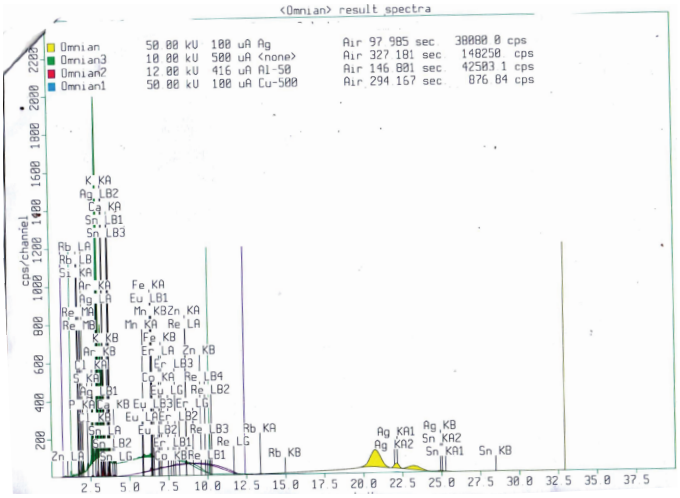

Fig. 6 - XRF Graph showing elemental content of red leaf lettuce

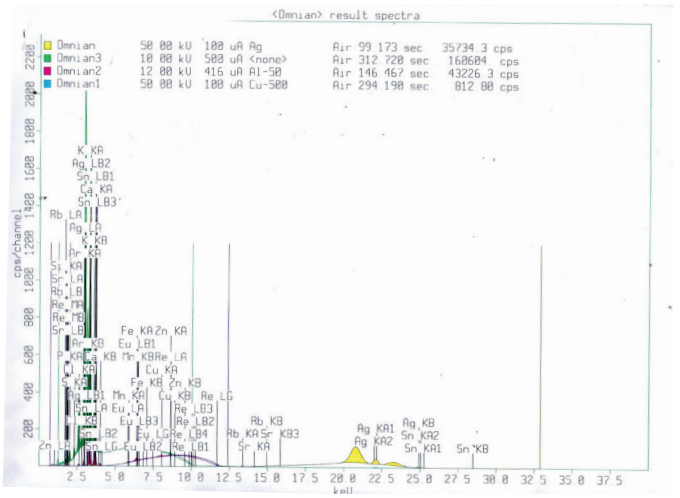

Fig. 7 - XRF Graph showing elemental content of green leaf lettuce

carotenoid using the following equation (Arnon, 1949).

Chl a (mg/ml) =12.25 ($A_{663.2}$nm) -2.79($A_{646.8}$nm)

Chl b (mg/ml) =21.50 ($A_{646.8}$nm)-5.10($A_{663.2}$nm)

Total chlorophyll (a+b): 7.15($A_{663.2nm}$) + 18.71 ($A_{646.8\ nm}$)

Carotenoid: [100(A_{470nm}) – (1.82 Chl a +85.02 Chl b] /198

X-Ray Fluorescence (XRF) analysis

The collected plants leaves were washed with tap water to remove mud and dust particles. About two gm. of this plants leaves were grinded with motor pestle by adding distilled water in proper ratio. The different elements present in the selected plants were investigated qualitatively by using X-ray Fluorescence (XRF) technique to investigate the chemical composition of different materials. XRF analysis was carried out at Department of Advanced Testing and Calibration Laboratory in Centurion University of Technology and Management using a handheld Epsilon 1 pananlytical XRF spectrometer.

RESULTS AND DISCUSSION

In this study estimating the chlorophyll content of leafy vegetables helped us to know the quantity of the pigments present in these leafy vegetables and thereby the nutritional requirements. In this study two varieties of *L. sativa* (green leaf and red leaf) were evaluated for their chlorophyll content. Chlorophyll and carotenoid estimation was done on the green and red fresh leaf samples extracted with the acetone and the absorbency readings of chlorophyll and carotenoid extracts were measured in 3 different wavelengths viz. 663.2nm, 646.8 nm and 470 nm respectively using spectrophotometer (Table 1). Based on the absorbency values (Table 2) calculations were made using Arnon's (1949) equation and the amount of chl a, chl b, total chlorophyll and carotenoid were estimated and tabulated (Table 3).

In two varieties of *L. sativa,* red leaf contained higher amount of Chl a and and Chl b with a value of 23.920mg/ml and 43. 207 mg/ml respectively than green leaf with Chl a and Chl b with 21.795 and 39.759 mg/ml respectively. The total chlorophyll content was highest in red leaf and estimated with 67.126 mg/ml than green leaf having 61. 552 mg/ml. The highest carotenoid content was estimated in green leaf with 260.41 mg/ml and the lowest carotenoid content was observed in red leaf with 246.01 mg/ml (Table 2, Fig.5). The possible justification could be that Chl a is the primary pigment while others pigments including Chl b are accessory pigments or because of low Fe content in soil might be the cause of low chlorophyll content **(**Srichaikul *et al*., 2011**).** It was reported from the recent study that chlorophyll rich diet help in preventing diseases (Kizhedath and Suneetha, 2011**;** Levent Inanc, 2011**).** This study suggest that it can be consumed as leafy vegetables in cooked or fresh state as salads to avoid in taking chlorophyll supplements to get health benefits.

From the Table 3 it was very clear that the total chlorophyll content in different days of seedlings of lettuce plant is increased along with increase of days up to 90 days of seedlings, but at the age of 61-90 days plants showing optimum content of chlorophyll. It is also found that the red leaf lettuce was found to contain more chlorophyll than the green leaf lettuce. The experimental resultant values support that the age of the plant does matter to the content of chlorophyll in lettuce plant. The chlorophyll content in plant directly relevant to nutrients like Nitrogen the maximum nutrient availability was detected within 61-90 days.

Data in Table 4 revealed the presence of 15 elements along with water content in red leaf lettuce and 14 elements and water in green leaf lettuce. Strontium and copper was found in red leaf variety where as in green variety these two elements were absent (Fig. 5 & Fig. 6). Er_2O_3 was estimated from green leaf varieties. Among all the nutrients, high amount of potassium was estimated in red varieties with 1.685% while green varieties contained 0.917% of potassium. About 95.914% and 96.83% of water was found in red and green varieties respectively. Water content was estimated more with 96.83% in green leaf lettuce than red with 95.91%.

High moisture content in these leafy vegetables will help

the water soluble enzymes and co-enzymes for metabolic activity (Iheanacho and Udebuani, 2009). It was also reported that the mineral composition of 7 different Nigerian leafy vegetables and reported high levels of Ca (63.36 to 110. 16 mg/ 100g), Mg (27. 51 to 288 mg/ 100g) and Na (15.01 to 88. 00mg/ 100g) respectively. Low levels of copper, nickel and manganese were also reported to be present in these leafy greens (Asaolu *et al*., 2012).

CONCLUSION

The concluded data can be useful to determine the proper harvesting time period for the lettuce plants and their consumption to get maximum nutritional benefits. The elemental composition was also varied according to the soil condition and according to the varieties. The high moisture content also provides for greater activity of water soluble enzymes and coenzymes needed for metabolic activities of these leafy vegetables. Chlorophyll content and presence of minerals and vitamins in these leafy vegetables will fulfill the dietary requirements.

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