

BIOCHEMICAL COMPOSITION OF PAK CHOI LEAFY STALK-

(BRASSICA CHINENSIS- SHANGHAI VARIETY)

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ABSTRACT:

Pak Choi play a significant role as a food, it is consumed as vegetable. It is rich in minerals like Manganese and Zinc. It is used as an important diet due to the high water and fiber content. The Pak Choi leafy Stalk (young stem) is a low moderate source of vitamins and minerals. This is directly related to the proportion of dark green leaves in the edible portion. In the preset study Pak Choi was purchased from the market, the stem extract was collected from half a kg of Pak Choi, grinded and extract was collected. This extract was analyzed for protein, inorganic phosphorus, copper, potassium, manganese, zinc and vitamin – C. Protein content was determined by Lowry's method. It was found to be 150 mg whereas leaves contain 1.5 g. Here the young stem lacks the protein content when compared to that of leaves of Pak choi. Electrolyte like Potassium was determined by flame photometry. The Potassium was found to be 130 mg. Vitamin – C was determined by redox titrimetrically. It was found to be 39.69 mg where as 45 mg of Vitamin –C has been reported in Pak Choi's green leaves. Copper was determined by spectrophotometry. Pak Choi's stem contains copper of 50 mg while its copper content has not been reported in leaf. The manganese and zinc were determined by complexometric titration. The zinc was found to be 28.944 mg where as the leaves contain less 0.19 mg of zinc. Hence the young stem is rich in Zinc. The manganese was found to be 5.40 mg where as the leaves contain 19 mg of manganese.

Keywords: Extract, Pok Choi, Titration, Manganese, Protein, Spectrophotometry.

INTRODUCTION:

Pak Choi (*Brassica Chinensis*) is a green annual plant of cruciferaceae family. It is one of the oldest and most popular leafy vegetable originated from the main land of China, Philippines, Vietnam and other East – Asian regions [1]. This humble cruciferaceae family leafy vegetable has captured the attention of the western world for its sweet, succulent nutritious leaves and stalks. Pak Choi is most often used for salads, although it is also seen in other kinds of foods, such as soups, sandwiches and wraps and burger [4]. It is one of the popular low calorie leafy vegetable. Nonetheless, it is a very rich source of many vital phytonutrients, vitamins, minerals and health benefitting antioxidants [18]. About 100 g of Pak Choi carries just 13 calories. It is one of the recommended vegetable in the weight reduction program falling under zero calories or negative calories [6, 7]. It has also gathered religious and

medicinal significance over centuries of human consumption. Pak Choi is being recognized more and more often for its standout nutrient richness [8]. There are different varieties of [2] Pak Choi: 1) Chinese white stalk , 2) Soup spoon type 3) Green stalk or shanghai, 4) Squat canton type [9]. When selecting the Pak Choi at the super market one has to choose darker greens over the light coloured ones. The darker the leaf higher the nutrient content [10]. Chinese white Pak Choi are steady plants with light to dark green leaves curl outwards [13]. The leaf stalks are white, broad short and flat. The plants tend to get about 1 foot high and are fairly fast growers. Soup spoon type, the leaves and stalk are thinner than the white type and they have better flavour. The leaves are slightly cupped with ladles. The stalks are semi-circular and have a tendency to overlap to each other at the bases and broaden out into a typical Chinese soup spoon type. This is a strong grower and tolerates heat well. In Green stalk Pak Choi or shanghai Pak Choi variety the leaf stalk is a light green colour and is flat and widen out at the base. The leaves usually are dark green and nicely rounded. It is a fast grower and is also fairly heat tolerant. It has a very good texture and flavour. This is the variety chosen in my work. Squat type or canton Pak Choi have short, dark green, slightly shuffled leaves and short, fat, white stalks. They are often harvested as ‘baby Pak Choi’, also known as Bok choy ranks very highly on the Aggregate Nutrient Density Index (ANDI) which rates food based not only on their vitamin and mineral content but also their phytochemical composition and antioxidant capacity [18]. Pak Choi has been found to possess certain, anti-cancer properties [15]. Several population studies have shown that people who eat more cruciferous vegetables have a lower risk of developing lung, prostate, colorectal and breast cancer. The glucosinolate found in these vegetables are converted into isothiocyanates in the body which helps the body to fight cancer [14, 19]. Pak Choi contains folate which plays a role in DNA synthesis and repair, thus preventing the formation of cancer cells from mutation in the DNA, vitamin C, vitamin A and beta-carotene function as powerful anti-oxidants that help to protect cells against free radical damage [20]. Selenium is a mineral that is not present in most fruits and vegetables, but can be found in Pak Choi. It plays a role in enzyme function and helps to detoxify some cancer causing compounds in the body [25]. Selenium prevents inflammation and also decreases tumor growth rates. The iron, phosphorus, calcium, magnesium, zinc and vitamin K in Pak Choi all contribute to building and maintaining bone structure and strength. Iron and zinc play crucial roles in the production and maturation of collagen. Though Phosphorus and Calcium are both important in bone structure, the careful balance of the two minerals is necessary for proper bone mineralization. Consumption of too much phosphorus with little calcium intake results in bone loss. Potassium, calcium and magnesium have been found to decrease blood pressure naturally. Maintaining a low Na intake is essential to lowering blood pressure, however increasing K intake may be just as important because of its vasodilation effects. According to the National Health and Nutrition Examination Survey, fewer than 2% of US adults meet the daily 4700 mg recommendation. Pak Choi’s folate K, Vitamin C and Vitamin B6 content coupled with its lack of cholesterol, all help to maintain a healthy heart. Vitamin B6 and folate prevent the buildup of a compound known as homo cysteine. When excessive amounts of homo cysteine accumulate in the body, it can damage blood vessels and lead to heart problems. Choline is a

very important and versatile nutrient in Pak Choi helps with sleep, muscle movement, learning and memory. Choline helps to maintain the structure of cellular membranes aids the transmission of nerve impulse assist in the absorption of fat and reduces chronic inflammation. The selenium found in Pak Choi has also been found to improve immune response to infection by stimulating production of killer T – Cells. Collagen, the skin's support relies on vitamin C as an essential nutrient [23]. Vitamin C also promotes collagen's ability to smoothen wrinkles and improve over all skin texture. Many studies have suggested that increasing consumption of plant food like Pak Choi decreases the risk of obesity, diabetes, heart disease and overall mortality while promoting a healthy complexion and hair, increased energy and overall lower weight. Pak Choi is popular winter season leafy vegetable in China and Korean parts. Its succulent leaves and stalks carry certain Antioxidants plant chemicals such as thiocyanates, indole – 3 – carbinol, lutein, zeaxanthin [22], sulforophanes and isothiocyanates that may offer protection against breast and prostate cancers [24].

In the present study we have choosen green stalk Pak Choi or shanghai Pak Choi. The stalk of the leaves were subjected for phyto chemical analysis as they are crispy, sweet and stalks can be eaten raw, added to salads, wraps, sandwiches and burgers. The stalk can be mixed well with cabbage in coleslaw. Hence the present investigation has been carried out to look for phyto nutrients in the young stalk of Pak Choi.

MATERIALS AND METHODS:

General chemicals and solvents used were of analytical grade procedure from RANKEM. They are casein, bovine serum albumin, sodium hydroxide, potassium chloride, Ce (IV), molybdate, liq. Ammonia, EDTA, Zinc sulfate, Folin's reagent, sodium carbonate, copper sulfate, triethanolamine, hydroxyl ammonium chloride, hexamine.

Collection of the extract from Pak Choi:

About 500 g of fresh green leaf Pak Choi (*Brassica Chinensis*) were obtained from the local market. Then leaves were cutoff and young stalk collected was grinded and from which 250 ml of the extract was obtained through filtration and centrifugation. The collected extract was stored in freezer and then the frozen extract was used for further analysis.

i) Protein content of Pak Choi extract by spectrophotometric method:

Protein content was carried out by Lowry's et al. method as described below [16]. It is the most commonly used method for determination of protein in cell free extract because of its high sensitivity and quantity as low as 20 μg of protein can be measured. The peptide bonds in polypeptide chain react with copper sulfate in an alkaline medium to give a blue colored complex. In addition tyrosine and tryptophan residues of protein cause reduction of the phosphomolybdate and phosphotungstate components of the Folin-Ciocalteau reagent to give bluish products which contribute towards enhancing the sensitivity of this method. However several compounds like EDTA, Tris, carbohydrates, thiol reagents, phenols etc. interfere with the colour development and it should be ensured that such substances are not present in

sample preparation. The experiment was conducted by adding a series of 0.05, 0.1, 0.15, 0.20, 0.25, 0.30 ml of 100 ppm BSA was taken in 25 ml calibration flask. In the similar way extract sample of about 0.1 and 0.2 ml was taken in the calibration flask and 1 ml of NaOH was added. To this add 5 ml of alkaline copper sulfate solution to each of the flask and allowed to stand for 10 minutes. Then add 0.5 ml of Folin's reagent and stirred well and allowed to stand for 10 minutes. The absorbance was measured at 340 nm. The graph was plotted as absorbance versus concentration of BSA. From the standard curve the amount of protein in the sample was determined.

ii) Determination of inorganic Phosphorus by spectrophotometry.

Phosphorus is one of the major nutrients required for better growth. It participates in the synthesis of important organic compounds such as phospholipids, ATP, Phosphoproteins, nucleic acid and other. Here the phosphorus can be determined by spectrophotometer [5, 17], which is based on Beer – Lambert's law. Phosphorus in Pak Choi can be very less and forms the complex in the form of phosphorus molybdate by reacting the phosphate ammonium vanadate and ammonium molybdate. This method is considered as slightly less sensitive than molybdate blue method but it has been particularly useful for phosphorus determination. The complex absorbance is measured at 480 nm, where the absorbed colour is blue and the transmitted colour is bright yellow. In to a series of 25 ml volumetric flasks 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml of standard phosphate solution was added through microburette. To each of the flasks (including test solution) 2 ml of ammonium vanadate solution, 2 ml of 2M HNO₃ solution was added to produce bright yellow colour of phosphovanadate molybdate complex. These solutions were allowed to stand for about 15 minutes. So that colour of the complex can fully develop. Each of these solutions was diluted up to the mark and the solution is shaken well for uniform concentration with distilled water. The % T of these solutions were measured at 480 nm using UV – Visible single beam spectrophotometer. A calibration graph of absorbance versus the concentration of standards was plotted. From which the concentration of inorganic phosphorus in the form of phosphate in the sample extract was calculated.

iii) Determination of copper by spectrophotometry.

Intensity of light decreases exponentially when the concentration of the substance increases arithmetically. Solution of CuSO₄ salt gives blue colour with ammonia and intensity of blue colour varies with the concentration of Cu²⁺ ion, when monochromatic light focused on a homogeneous medium. It is partly reflected, partly absorbed and partly transmitted. Using spectrophotometer optical density is determined for different concentration of Cu²⁺ ion and unknown solution of Cu²⁺ ion. A graph of optical density versus concentration gives straight line passing through origin from which concentration of copper in the extract sample can be determined. About 0.1 M solution of copper sulfate was prepared and 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5 ml of the solution was run down into 6 different 25 ml standard flask. About 5 ml of liquor ammonia was added to each flask to get cuprous ammonium sulfate solution and made up to the mark with distilled water. Similar procedure is carried out with the unknown

amount of extract sample. By using any one of the solution the % T of the solution at different wavelength was calculated. Then the % T was determined using the solution of different concentration prepared. A calibration graph was constructed by plotting the absorbance versus concentration of copper sulfate from which concentration of the copper in the extract sample was calculated.

iv) Determination of potassium by flame photometry

Flame photometry is used primarily for the analysis of elements which have an easily excited flame spectrum of sufficient intensity in the detection by a photocell [5]. The region of the spectrum appropriate to the element being determined is isolated by means of optical filters. The method of excitation of KCl in the flame is as follows. The water (or the solution) evaporates, leaving behind minute particles, particles of the salt or mixture of salts. At higher temperature of the flame KCl decomposes and vaporizes to give constituent atoms. The vapours of potassium are then excited by the thermal energy of the flame. Finally the resulting emission spectrum is got. Potassium when exposed to flame shows lilac colour. 1, 2, 3, 4, and 5 ml of 100 ppm standard potassium solution was pipette into a series of 25 ml of volumetric flask so as to get the concentration of the solution 4, 8, 12, 16 and 20 ppm respectively and diluted up to the mark with double distilled water and shaken well. All the solutions were analysed in the flame spectrometer and readings were recorded. Extract sample was also analysed in the same way. A graph was drawn by plotting concentration of potassium against meter reading, from the plot concentration of potassium in the extract sample was determined.

v) Determination of Manganese using Eriochrome Black-T as an indicator

About 5 ml of the sample extract solution was pipette into a clean conical flask. About 0.5 g of hydroxyl ammonium chloride (to prevent oxidation). Warmed and diluted to 100 ml with boiled out distilled water. If the solution is acidic, neutralized with dilute sodium hydroxide solution. About 3 ml of triethanolamine added to keep the manganese in solution. When it was subsequently made alkaline, 2 ml of buffer solution (pH=10), and several drops of Eriochrome Black-T indicator was added. Titrated with standard 0.05M EDTA until the colour changes from red to blue [21]. The amount of manganese present in the sample extract was calculated using the relation, 1 ml of 0.05M EDTA= 27.47 mg of Mn.

vi) Determination of zinc using xylenol orange as an indicator.

About 5 ml of the extract sample was taken and pipette into a clean conical flask. Diluted with 10 ml of distilled water and 3 drops of xylenol orange indicator. A spatula of powdered hexamine added with agitation to the resulting yellow solution until it acquires an intense red colour. Then titrated with the standard EDTA solution until the colour changes sharply from red to yellow [21]. The amount of zinc present in the extract sample calculated using the relation. 1 ml of 0.05M EDTA = 3.769 mg of zinc.

vii) Determination of vitamin-C by redox titration.

Vitamin-C can be estimated by titrating it against Cerium(IV) using Ferroin as an indicator. Being a strong oxidizing agent Ce(IV) oxidizes vitamin-C in an acidic medium to its hydroform. 10 ml of 0.01M FAS was pipette into a clean conical flask. 2 ml of 2M H₂SO₄ was added and titrated against Ce(IV) solution taken in the burette using Ferroin as an indicator. Titration carried out till the colour changes from orange to pale green. Titration was repeated to get concordant values. Titer values were recorded and concentration of Ce(IV) solution was calculated. 5 ml of the extract sample was pipette into a clean conical flask, 2 ml of H₂SO₄ was added and titrated against standard Ce(IV) solution, taken in the burette till the colour changes from red to pale green using Ferroin as an indicator. Titration was repeated to get concordant values and amount of vitamin-C present in the extract was calculated.

RESULTS AND DISCUSSIONS:

Pak Choi play a significant role as a food, it is consumed as vegetable. As a vegetable it is rich in minerals like Manganese and Zinc. It is used as an important diet due to the high water and fiber content. The Pak Choi's stalk is a low moderate source of vitamins and minerals. This is directly related to the proportion of dark green leaves in the edible portion.

In the preset study the extract from the Pak Choi's young stalk was collected from 0.50 kg of Pak Choi, from that 0.5 kg of Pak Choi's young stalk was grinded and extract was collected. This extract was analyzed for protein, inorganic Phosphorus, copper by spectrophotometric method, potassium by flame photometric, manganese and zinc by complexometric and vitamin C by redox titration. Protein content was determined by Lowry's method. It was found to be 150 mg whereas leaves contain 1.5 g [23]. Here the young stalk lacks the protein content when compared to that of leaves of Pak Choi as shown in the table 1.

Table 1: Protein content of Pak choi young stem sample

Sl. No	Protein solution (std)	Conc. in ppm	Vol. of NaOH in mL	Alkaline CuSO ₄ solution mL	Foling's reagent	Incubation time (mins)	Absorbance at 660 nm
1	0.05	2	1	5	0.5	10	0.011
2	0.1	4	1	5	0.5	10	0.023
3	0.15	6	1	5	0.5	10	0.035
4	0.2	8	1	5	0.5	10	0.047
5	0.25	10	1	5	0.5	10	0.055
6	0.3	12	1	5	0.5	10	0.067

7	Sample 1 (stalk)	-	1	5	0.5	10	0.021
8	Sample 2 (stalk)	-	1	5	0.5	10	0.033

Table 2: Determination of inorganic phosphorus using spectrophotometry.

SL. No.	Volume of Phosphate Solution (1000 ppm)	Concentration of Phosphate in ppm	Volume of amm. vanadate Solution	Volume of 2 M HNO ₃ Solution	% T	A = 2 - log T
1	0	0	2	2	100	-
2	0.5	20	2	2	80.7	0.0931
3	1	40	2	2	65.3	0.185
4	1.5	60	2	2	53.9	0.2684
5	2	80	2	2	43.9	0.3575
6	2.5	100	2	2	36.1	0.4424
7	Sample 1 (Stalk)	-	2	2	42.7	0.3095
8	Sample 2 (Stalk)	-	2	2	25.4	0.5867

The phosphorus was determined by spectrophotometry. It was found that the young stalk contains 16.10 mg of inorganic phosphorus which is as shown in the table 2, whereas the leaves of Pak Choi contain 37 mg of phosphorus.

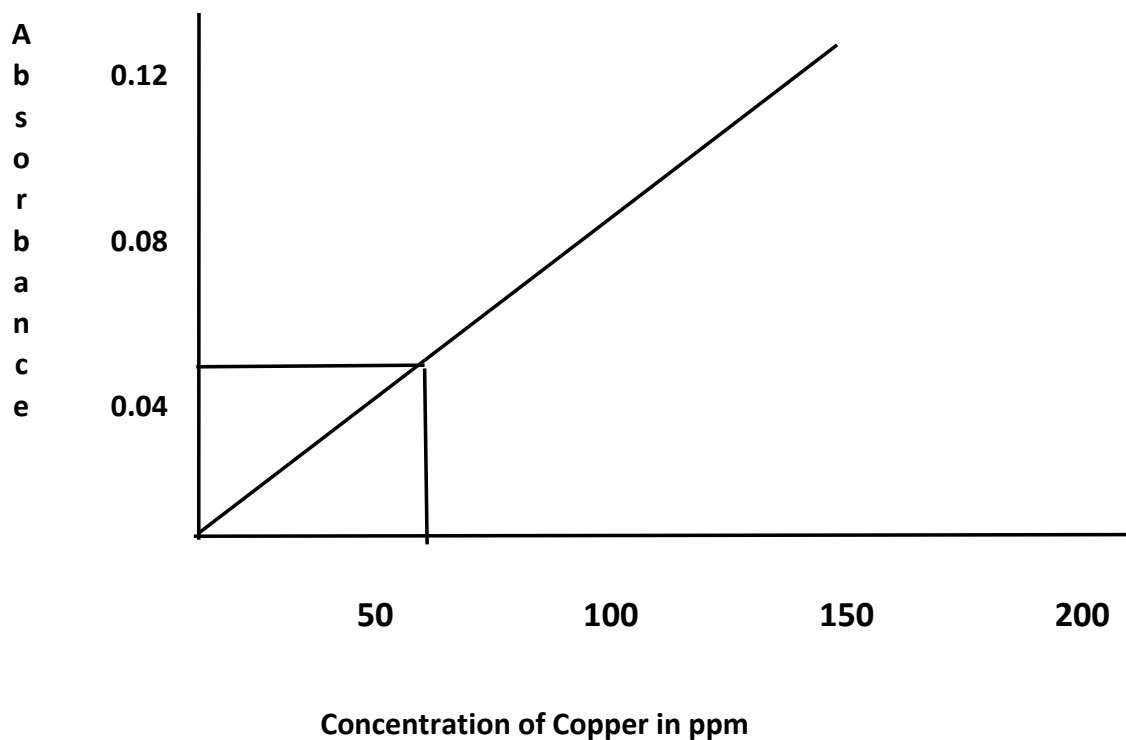


Fig 1: Concentration of copper using spectrophotometry:

Table 3: Determination of Potassium using flame photometry.

SL. NO.	Volume Of KCl in ml	Concentration of Potassium in ppm	Meter Reading
1	1	4	3
2	2	8	7
3	3	12	12
4	4	16	16
5	5	20	20
6	Sample	-	11

Pak Choi stalk contains Copper of 50 mg while its copper content has not been detected in leaf. Copper was determined by spectrophotometry as shown in figure 1. Electrolyte like potassium was determined by flame photometry. The potassium was found to be 130 mg and it is shown in table 3.

Table 4. Comparison of Biochemical Components in Pak choi leaves and young stem Extract

Sl. No	Nutrients	Pak Choi leaves/100 g (literature) in mg	Pak Choi Stalk/100 g (experiment) in mg	% less in stalk
1	Protein	1500	150	10
2	Phosphorus	37	16.1	43.51
3	Copper	-	50	-
4	Potassium	252	130	51.58
5	Manganese	19	5.4	28.42
6	Zinc	0.19	28.94	-
7	Vitamin C	39.69	45	-

The manganese was also determined by complexometric titration. The manganese was found to be 5.40 mg where as the leaves contain 19 mg of manganese. The zinc was determined by complexometric titration. The zinc was found to be 28.944 mg where as the leaves contain 0.19 mg of zinc. Hence the young stalk is rich in zinc. Vitamin C was determined by redox titration. It was found to be 39.69 mg where as 45 mg of vitamin C has been reported in Pak Choi's green leaves.

In the present investigation the zinc was found to be 28.944 mg in the stalk compared to 0.19 mg in leaves. Like all leafy vegetables, Pak Choi contains zero calories. It is rich in precious nutrients like zinc, vitamin C etc in both leaf as well as stalk as shown in the table 4.

Table 5. Comparison of Biochemical Components in Pak choi stem and Rhubarb extract

Sl. No	Nutrients	Pak Choi Stalk/100 g (literature) in mg	Rhubarb extract(experiment) /100 g in mg	% more in Pak Choi stalk
1	Protein	150	950	-
2	Phosphorus	16.1	8.8	45
3	Copper	50	0.68	98
4	Potassium	130	-	-
5	Manganese	5.4	12.6	-
6	Zinc	28.94	0.16	99
7	Vitamin C	45	10.8	24

The above contents were also compared with Rhubarb. It is also a very rich source of many vital phyto-nutrients and health benefiting antioxidants. but compared to composition of pak choi stalk has more % of inorganic phosphorus, copper about 98% more, zinc with 99% more, vitamin C with 24% more as shown in table 5 [12]. Hence along with pak choi leaves young stem/stalk also should be consumed for more nutrients. Hence the above investigation was carried in order to explore the importance of phyto nutritional composition of Pak Choi.

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