Indian J. Applied & Pure Bio. Vol. 40(1), 129-138 (2025). **ISSN: 0970-2091**
A Web of Science Journal

Biochemistry or chemistry and biology interface Biochemical study of raw and steamed extract of Chinese cabbage-*Brassica pekinensis* and comparison with *Brassica chinensis*

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Abstract

Chinese cabbage, also known as napa cabbage, is a leafy vegetable native to China, member of the Brassica family, good source of vitamins, minerals and linked to a number of health benefits. It is a good source of fiber, helps to improve digestion and promote regularity in the gut and also good choice for people to lose weight because of low calories, low fat, cholesterol and prevents cardio vascular diseases. In the present investigation, 1.0 kg of napa cabbage was collected from super market; 500g raw was grinded in sodium acetate buffer of pH 4.5, extract was collected. Another 500g was steamed for 10 mins at 100 ^{O}C, extract collected. Both the extracts were subjected for analysis of biochemical composition such as iron(ll), iron(lll), phosphorus, proteins, copper, by spectrophotometric method, reducing sugar (glucose), vitamin-C by redox titration, calcium, magnesium, manganese, and zinc by complexometric titration. Iron(II), phosphorus, protein, reducing sugar, vitamin C, calcium, magnesium, manganese and zinc contents have been drastically reduced by 10%, 25.58.0%, 25.6%, 35%, 86.64%, 62.8%, 40.5%, 6.0% and 38.0% respectively in steamed sample. Comparison has also been made with other species of *Brassica chinensis*. Iron content found to be more in steamed sample, copper remains same. But, other contents have been drastically reduced in steamed sample. Hence better to be consumed as salads instead of cooking.

Key words: Cabbage, nutrition, composition, spectrophotemetry, titrimetry.

Chinese Cabbage, also known as napa cabbage, is a leafy vegetable, native to China. It belongs to Brassica family, broccoli, cauliflower, kale and pak choi are other species which comes under this family. Chinese cabbage is widely cultivated in East Asia and has become increasingly popular in different parts of the globe. Its botanical name is *Brassica pekinensis*. Chinese cabbage is known for its long, cylindrical heads and white

inner leaves. It has a mild, sweet flavor and a crisp texture, making it popular vegetables for salads, stir-fries and soups. Varieties of Chinese cabbage are available[19]. Michihili variety is known for its large, heavy heads and long storage life. Green tower variety has a more slender head than michihili and is more resistance to bolting[17]. Spring, early-maturing varieties are well-suited for spring and fall planting. Chihili variety is popular in Korea for making kimchi. In addition to the main cultivar groups (napa cabbage and chinensis pak choi), there are also a number of other varieties of Chinese cabbage[7], including Wombok- variety is a cross between napa cabbage and bok choy (pak choi). It has a long, cylindrical head and thick white stalks. Choy Sum variety has long, slender leaves and yellow flowers. It is often used in stir-fries and soups. Mizuna variety has long, serrated leaves and a mild flavor. It is often used in salads and garnishes. Chinese cabbage can be either direct seeded or raised as transplants[28]. Napa cabbage is typically larger than bok choy, with heads that can reach upto 12 inches in diameter and 18 inches in height[4,26]. The leaves of napa cabbage are long and slender, with a crinkly texture. Chinese cabbage, also known as napa cabbage, thrives in well-drained, fertile soil with acidic to neutral environment ensures optimal nutrient availability for the plant's growth and development[13].

Chinese cabbage has numerous health benefits. It carries good amount of vitamin-C, involves in improving the immune capacity. Chinese cabbage contains glucosinolates, which are compounds that have been shown to protect against cancer. It is also rich in potassium, which helps to regulate blood pressure, also rich in fiber, which helps to improve digestion and promote regularity, low-calorie food and aids in weight loss. It is a very good source of beta carotene, known to protect against cataracts and macular degeneration. It is a good source of iron[27], which helps the body to increase haemoglobin level in the blood.

Studying Chinese cabbage, also known as napa cabbage, is crucial for various reasons. It has great nutritional significance[23]. Chinese cabbage is a rich source of essential nutrients[14], not only vitamins, but also contains minerals like potassium, magnesium, nitrates and nitrites[8]. It possesses a variety of phyto-chemicals, including glucosinolates, flavonoids, carotenoids, which have been linked to potential health benefits[15]. It gains much attention as agricultural crop. Chinese cabbage is a globally important crop, ranking among the top five most consumed vegetables worldwide[5]. It is relatively easy to cultivate and adapts well to various climates, making it a valuable crop for food security and economic development. Chinese cabbage is a versatile ingredient in many cuisines, particularly Asian cuisine. Its mild flavor, crisp texture, and high water content make it suitable for various culinary preparations, including salads, stir-fries, soups, and kimchi. Genetic and Molecular Understanding of Chinese cabbage is gaining much attention. Understanding the genetic makeup and molecular mechanisms underlying Chinese cabbage's traits, such as head formation, nutrient content, and stress tolerance, can aid in breeding improved varieties and developing sustainable production practices[29]. Studying Chinese cabbage's adaptability to different environmental conditions and developing improved cultivars with enhanced traits like

disease resistance, pest tolerance, and yield potential are crucial for sustainable agriculture. Investigating effective methods for preserving and processing Chinese cabbage, such as pickling, fermentation and freezing, can extend its shelf life, reduce food waste, and enhance its availability. Research suggests that Chinese cabbage keeps away serious ailments including cancer and heart disease, potential benefits for bone health and cognitive function. Because of its numerous health benefits, the present work has been undertaken to investigate its extract for important biochemical composition.

Chemicals were of AR grade purchased from SRL Chemicals. It includes casein, Bovine Serum albumin (BSA), Folin's reagent, sodium hydroxide, sodium carbonate, copper sulphate, ammonium ferrous sulphate, $CeSO_4$(IV), $ZnSO_4$, Ethylene Diamine Tetra Acetate (EDTA), ammonium chloride, potassium dihydrogen phosphate, molybdate, sodium sulphite, sodium hydrogen sulphite, sodium chloride, potassium chloride, potassium hydroxide etc.

Extract (sample) preparation from Chinese cabbage :

About 1Kg of Chinese cabbage was collected from the super market. Around 500 g of it was grinded with sodium acetate buffer at pH 4.5 and extract was collected. The sample was analysed for nutrients such as iron(ll), iron(lll), copper, proteins, phosphorus by spectrophotometric method, reducing sugar (glucose), vitamin-C by redox titration, calcium, magnesium, manganese, and zinc by complexometric titration.

Determination of Iron(II) by spectrophotometry

Iron(II) reacts with 1, 10 – phenanthrolein. The coloured complex formed was determined by spectrophotometer. The spectrum is plotted to determine the absorption maximum. Hydroxylamine hydrochloride is added to reduce Fe^{3+} to Fe^{2+}. Into a series of 25 milliliter volumetric flask, add with pipette 0.50, 1.0, 1.5, 2.0 and 2.5 milliliter of 100 ppm solution so as to get the concentration of 2, 4, 6, 8 and 10 ppm respectively[6]. Add 2 milliliter of 1,10-phenanthroline and 3 milliliter of buffer in each of the flask. The absorbance readings were recorded at 510 nm. Sample reading was also measured in the same way, plotted a calibration curve and concentration was determined.

Estimation of Iron(III) by spectrophotometry:

Fe^{3+} reacts with ammonium thiocyanate to give red coloured complex, being stable in the solution. Calibration curve was prepared by pipette, 2, 3, 4 and 5mL of 50 ppm solution a series of 25 mL of volumetric flask, so as to get the concentration of solution 2, 4, 6, 8 and 10ppm respectively[21]. Add 2.0 mL of thiocyanate and 3mL HCl in each of the flask. The absorbance of the solution was read at 480 nm. A standard curve was plotted with the concentration in ppm on X-axis, absorbance of the solution was recorded and entered in the table.

Determination of inorganic phosphorous by spectrophotometry :

Ammonium phosphomolybdate is formed when inorganic phosphorus reacts with ammonium molybdate. This is reduced with a

mild reducing agent to produce molydenum blue. The blue colour of the solution is measured spectrophotometrically[6] at 480 nm. To 10 milliliter of standard flask pipette out 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL of 1000 ppm phosphate solution, to each of the flask 0.8 mL of ammonium vanadate solution and 1mL of 2.5M HNO_3 solution were added, kept a side for about 15 min. So that color of complex can be developed. Make up the solution using double distilled water, similarly the extract was taken and 0.8 mL of ammonium molybdate and ammonium vanadate, 1.0 mL 2.5 N HNO_3 was added, absorbance of each solution recorded at 480nm. The concentration of phosphate in samples were determined with the help of calibration curve. A calibration graph was constructed and concentration was determined in the extract sample.

Estimation of Protein by spectrophotometry:

Lowry's method is the highly sensitive and available method for the determination of protein[20]. The experiment was conducted by adding a series of 0.05, 0.01, 0.15, 0.20, 0.25, 0.30, 0.35, 0.4mL of BSA was taken in 25mL standard flask. To this solution NaOH was added to make the volume 1mL. In the similar way extract of about 0.1 and 0.2 mL was taken in the standard flask and 1mL of NaOH was added. To this add 5mL of alkaline Copper sulphate solution to each flask and allowed for 10 min incubation. Then 0.5 mL of Folin Ciocalteau reagent was added and stirred well, incubated for 10 min, the absorbance was read at 660 nm. The graph was plotted to determine the amount of protein in the sample.

Determination of copper by spectrophotometry

In this experiment optical density is determined for different concentration of Cu^{2+} ion and unknown solution of Cu^{2+} ion. Into 25 ml standard flask, 0.1 M copper sulphate solution was dispensed with 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5 ml, followed by 5 ml of liquor ammonia to get cuprous ammonium sulphate 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 standard graph was drawn by plotting the absorbance on Y-axis and concentration of copper sulphate on X- axis from which amount of the copper in the sample was calculated.

Determination of amount of reducing sugar (glucose) :

The method involves the boiling of measured volume of sugar solution with excess of alkaline copper hydroxide, the precipitate cuprous oxide formed, dissolved in ferric alum, the formed ferrous by the reducing sugar is titrated with known permanganate solution. About 10mL of sample solution was pipette out and 20 mL of $CuSO_4$ solution, 20mL of alkaline tartrate and 15mL of H_2O was added, the mixture is boiled and allowed the precipitate to settle down. Then filtered the above solution using alien filter. The precipitate cuprous oxide is collected and filtrate is rejected. The precipitate is dissolved using acid ferric sulphate. The filtrate is titrated with known concentration of $KMnO_4$ solution till the solution turns permanent pale pink. From the titer value, the amount of glucose was deduced.

Determination of vitamin – C by Redox titration :

It was determined by redox titration using cerium sulphate as titrant. It was standardised using 0.01M FAS using ferroin indicator. About 2.0 mL of Chinese cabbage extract was pipette into the conical flask, titrated against standard cerium sulphate solution. Few drops of ferroin indicator was added. Titration was repeated to get concordant value and amount of Vitamin –C present in the Chinese cabbage sample was calculated.

Estimation of calcium and magnesium by EDTA titration :

EDTA being promising titrant was standardized using $ZnSO_4.7H_2O$ (Day and Underwood). About 0.01 M standard $ZnSO_4.7H_2O$ was titrated against EDTA, solution turns wine-red to blue. Repeat the titration for concordant values. Pipette out 5 mL of the extract solution, dilute it to 10mL dist. H_2O and add 3 mL of buffer solution titrate against EDTA, till the colour from wine red to blue. Note down the values, which determine concentration of both calcium and magnesium in the extract. The amount of Ca and Mg in the sample was deduced with the relation 1mL of 1M EDTA = 100 milligram $CaCO_3$.

Determination of calcium alone: About 5 mL of the extract solution was pipette into conical flask, dilute it to 10mL and 2mL of 8M KOH solution titrate against EDTA using Patton Reeder's indicator. The concentration of calcium alone in the sample was deduced with the relation 1mL of 1M EDTA = 40 milligram of Ca.

Determination of Manganese :

Manganese ions (Mn^{2+}) in a sample was estimated by EDTA titration (Day and Underwood). To 5mL of the extract 0.5 g of hydroxyl ammonium chloride was added (to prevent oxidation). Warmed and diluted to 100 mL with boiled distilled water (if solution acidic, neutralised by using dilute sodium hydroxide solution). About 3mL of tri ethanol amine is added to keep the manganese in solution, and it was subsequently made alkaline by adding 2mL of buffer solution (pH-10) and several drops of EBT indicator was added. The contents were titrated with 0.01M EDTA. The concentration of manganese in the sample was deduced with the relation 1mL of 1M EDTA = 27.47 milligram of Mn.

Determination of Zinc by Complexometric titration :

Zinc being an important nutrient for immune and brain function, also crucial for nervous system, blood sugar and optimal health. It was determined by EDTA titration. To 5 mL of the sample taken in the conical flask, 10 mL of distilled water and 3 drops of indicator xylenol orange, a pinch of powdered hexamine added with constant stirring. Yellow solution turns red color[25]. This solution was titrated with the standard EDTA solution. The concentration of zinc present in the extract sample calculated using the relation 1mL of 1M EDTA = 65.38 milligram of zinc.

In the present study, iron(ll) and iron (lll) was determined by spectrophotometry. It was found that raw Chinese cabbage contains 0.5mg /100g and 0.3mg/100g where as steamed Chinese cabbage contains 0.45mg/100g and

0.5mg/100g respectively as shown in the table 1. It was observed that the amount of iron(ll) was reduced by 10% in steamed extract where as iron(lll) is more in steamed sample of Chinese cabbage (Table-5). But around 60% and 8.3% has been lost in *Brassica oleracea*[11]. Phosphorus was determined by spectrophotometry and was found that raw Chinese cabbage contains 1.075mg/100g where as steamed Chinese cabbage contains 0.8mg/100g (Table-2). It was observed that while steamed the amount of Phosphorus has reduced by 25.58, while 16% has been noticed in *Brassica oleracea*[11]. Protein was determined by Lowrys method and was found that raw extract contains 0.86 g/100g where as steamed extract contains 0.64 g/100g, 25.6% has been reduced on steaming (Table 3). But in *Brassica chinensis* more protein is found in raw extract[12]. Copper determined by spectrophotometry was found that raw sample contains 0.199mg /100g where as steamed extract contains 0.20mg/100g. It was observed that the amount of copper remains same even on steaming (Table-4). Copper has not been detected in *Brassica chinensis*. Glucose was determined by Bertrand's method was found to be 3.098g/100g in raw Chinese cabbage and in steamed Chinese cabbage 2.017g/100g. It was observed that while steamed the amount of glucose has reduced by 35% (Table-5). Almost same glucose content has been observed in *Brassica chinensis*. Ascorbic acid was examined by redox titration, results revealed that raw Chinese cabbage contains 25mg/100g where as steamed Chinese cabbage contains 3.34mg/100g. It was observed that while steamed, the concetration of Ascorbic acid has decreased by 86.64% exhibiting thermolabile property. Compared to pkinensis, chinensis has more vitamin C content. Ca, Mg, Mn and Zn were examined by EDTA titration revealed that raw extract contains 75mg, 12mg, 14.8mg and 49.4mg /100g respectively, where as steamed sample contains 27.9mg, 7.14mg, 13.88 and 30.6mg/100g respectively. It was observed that while steamed extract exhibited calcium, magnesium is drastically reduced by 62.8 and 40.5%, manganese and zinc by was reduced by 6 and 30.6% .

It was found that steamed sample has lost some percentage of nutrients being vitamin C and calcium more. But the copper content almost remains same in both samples. Hence, it can be eaten raw, cooked or fermented as

Table-1. Determination of Fe^{2+} in Chinese cabbage sample

Volume of Fe(II) in mL	Concentration of Fe(II) in ppm	Volume of 1,10-phenanthroline in mL	Volume of Buffer in mL	Absorbance
0.4	2	2	3	0.160
0.8	4	2	3	0.335
1.2	6	2	3	0.515
1.6	8	2	3	0.680
2.0	10	2	3	0.871
Sample -1	-	2	3	0.322
Sample -2	-	2	3	0.559

Table-2. Determination of phosphorous in Chinese cabbage sample

Volume of solution in mL	Concentration of Phosphorus in ppm	Volume of ammonium molybdate in mL	Volume of ammonium vanadate inmL	Volume of 2.5M HNO_3 in mL	Absor-bance
0.2	20	0.8	0.8	1	0.103
0.4	40	0.8	0.8	1	0.158
0.6	60	0.8	0.8	1	0.211
0.8	80	0.8	0.8	1	0.283
1.0	100	0.8	0.8	1	0.358
1.2	120	0.8	0.8	1	0.399
Sample-1	-	0.8	0.8	1	0.175
Sample-2	-	0.8	0.8	1	0.320

Table-3. Determination of protein in Chinese cabbage

Volume of BSA in mL	0.1N NaOH in mL	Reagent alkaline $CuSO_4$ in mL		Follin's reagent in mL		Absor-bance
0.05	0.95	5		0.5		0.164
0.1	0.90	5		0.5		0.250
0.15	0.85	5	Mix and	0.5	Keep it	0.352
0.20	0.80	5	Keep it	0.5	For 30	0.394
0.25	0.75	5	For 10	0.5	minutes	0.471
0.30	0.70	5	minutes	0.5		0.534
0.35	0.65	5		0.5		0.666
0.40	0.60	5		0.5		0.755
Sample-1	0.80	5		0.5		0.389
Sample-2	0.70	5		0.5		0.541

Table-4. Determination of Copper in Chinese cabbage sample

Volume of $CuSO_4$ solution in mL	Volume of liquid ammonia in mL	Absorbance at 610nm
1.25	5	0.017
2.5	5	0.024
3.75	5	0.032
5.0	5	0.043
6.25	5	0.056
7.5	5	0.063
Sample -1	5	0.008
Sample -2	5	0.022

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Table-5 Comparison of nutritional value between raw and steamed extract of *Brassica pekinensis* and *Brassica chinensis*

Nutrients	*Brassica Pkinensis* (Napa Cabbage)			*Brassica chinensis* (pak choi)
	Raw extract in mg/100 g	Steamed extract in mg/100g	% loos of nutrients on steaming	Raw extract in mg/100 g
Iron(II)	0.5	0.45	10	4.66
Iron(III)	0.3	0.5	more	5.67
Phosphorous	1.075	0.8	25.58	37
Copper	0.199	0.2	same	-
Proteins	860	640	25.6	1500
Reducing sugar	3098	2017	35	2190
Vitamin C	25	3.34	86.64	40
Calcium	75	27.9	62.8	28.61
Magnesium	12	7.14	40.5	5.4
Manganese	14.8	13.88	6	19
Zinc	49.4	30.6	38	0.19

most popular ingredient of stir- fries, salads, soups, kimchi and steamed dishes. It is rich in vitamins, fiber and minerals like potassium and it offers various health benefits. Therefore the present study was undertaken to reveal the significance of biochemical composition of Chinese cabbage.

In this work raw and steamed sample obtained from 1000 g of Chinese cabbage were analysed for biochemical composition such as iron(ll), iron(lll), phosphorus, proteins, copper, by spectrophotometric method, reducing sugar (glucose), vitamin-C by redox titration, calcium, magnesium, manganese, and zinc by complexo-metric titration. Some % of loss in the nutrients on steaming has been found. Therefore Chinese cabbage can be eaten raw or fermented. It is a popular ingredient in stir-fries, soups, salads, kimchi. Brassica pkinensis is a nutritious and act as a versatile plant that can be enjoyed in a variety of dishes, being rich in vitamins and minerals imparting various health benefits. It is a popular ingredient in many Asian cuisines and is known for its mild flavour and crisp texture. Hence this study was undertaken to exhibit the nutritional benefits of *Brassica pekinensis*.

I wish to acknowledge my sincere and heartfelt thanks to the Head of the institution, MSCW, Mysuru-570005, Karnataka, India for his constant and consistent support throughout this work.

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