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A BIOCHEMICAL APPROACH TO COMPARATIVE EVALUATION OF RAW AND COOKED ZUCCHINI EXTRACT

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Abstract

Cucurbita pepo, often known as the zucchini courgette or baby marrow, is an annual herbaceous summer squash that is rich in vitamins, minerals, and health-promoting chemicals. Water and fiber, which are abundant in zucchini, can aid in promoting a healthy digestive system by lowering symptoms of constipation and other gut-related illnesses. In the present study, 500g of Zucchini was grinded and extract was collected. Another 500g was cooked for 10 mins at 100 ^{0}C, extract collected. Both extracts were used for analysis of nutrients. Zinc and Manganese content in raw and cooked extract was carried out by complexometric titration and was found to be 1.01, 1.02 and 0.150, 0.143 mg/100g respectively. The spectrophotometric approach yielded an iron(III) content of 0.238 and 0.119 mg/100g for the raw and cooked samples, respectively. The protein determined by in raw and cooked extract was 0.529 and 0.09 mg/100g has been found. The reducing sugar determined was found to be 3.18 and 0.10 mg/100g in raw and cooked extract. Zinc, manganese, sugar and protein contents have been drastically reduced by 85%, 86%, 97% and 83% respectively in cooked sample. Hence it can be consumed as salads like cucumber instead of cooking. Hence the present investigation was undertaken to study its nutritional composition.

Keywords: Zucchini, Spectrophotometry, Cooked, Nutritional composition.

INTRODUCTION

A summer squash, zucchini (also known as courgette) is harvested when it is still immature and fresh, with a soft, edible rind. A yellow squash that is available in the summer is sometimes referred to as summer squash (Bombardelli). The most common variety of summer squash is really the green one called zucchini. It was produced by a mutation in

nature. Unlike pumpkins, the skin or peel of these two types of squash is easily edible and has a soft shell. The exact origin of zucchini is uncertain, it is believed to have originated in central and south America, and there is a brief history of zucchini. zucchini's popularity spread to various parts of the world, including other European countries, Australia and parts of Asia. Today, it is cultivated and consumed in many regions worldwide and has become a beloved vegetable globally, valued for its mild flavour, versatility in cooking, and its nutritional profile (USDA). It continuous to be a prominent ingredient in many traditional and modern recipies, showcasing its journey from the ancient civilization of the Americas to its widespread presence in today's culinary landscape.

Zucchini belongs to the species *Cucurbita pepo*, which is a part of the Cucurbitaceae family. Cucurbitane glycosides and triterpenoid chemicals are found in substantial quantities in all four types of squash (acorn, crooked neck, straight neck, and pumpkin) in the genus Cucurbita (Radford). An annual herbaceous plant is zucchini. It has a trailing or climbing habit, with long, thin vines that spread out on the ground or can be trained to grow on supports. The fruit of zucchini is classified as a summer squash. It is typically harvested when young and tender, before the rind becomes high. The fruit has a cylindrical shape with smooth, thin skin. It comes in various shades of green, from pale to dark green and sometimes with mottled patterns. The edible part of zucchini is fruit. It is usually harvested and consumed before the seeds fully develop. The flesh of zucchini is pale green or white and has a mild, slightly sweet flavour.

Zucchini has four primary kinds. They're Crook neck refers to a squash that has a bent "neck" and a rounder "body." A more cylindrical squash with a small, straight "neck" is referred to as having a "straight neck." According to Sánchez-Meseguer and Sánchez-Mata, pomo is a long, cylindrical squash with light green skin and somewhat flavorful white flesh. The disk-shaped patty pan has scalloped sides and is renowned for its buttery flavor. Smaller, green, toy-top shaped squash with a sweet and spicy flavor is called a scaloppini. Sunburst: a little, toy-top-shaped, bright yellow squash with a green patch near the blossom end.

Zucchini is a great vegetable as it is low in calories and high in vitamins, minerals, and nutritional fiber. It is especially high in potassium, folate, vitamin C, and vitamin A. To further add to its health benefits, it contains polynutrients and antioxidants. Zucchini has health benefits and is high in nutrients (Mohammad et al.). According to Martinez-Valdivieso et al., zucchini is a rich source of numerous nutrients, including a range of vitamins, minerals, and healthy plant components. While raw zucchini has somewhat less vitamin A than cooked zucchini, cooked zucchini has a higher vitamin A content. Numerous antioxidants found in zucchini may provide a range of health advantages (Blum et al., Ismail et al., and El-Sayed et al). The fruit's peel has the highest concentrations (Oloyede et al). As a result of its high water content, the USDA National Nutrient Database states that it has little calories. Packed with fiber and other nutrients that can help support a healthy digestive system by lowering symptoms of constipation and other gut diseases. The fiber in zucchini may improve blood sugar stability and insulin sensitivity, which may lower the risk of type 2 diabetes. Zucchini's fiber, potassium, and carotenoids may reduce blood pressure, cholesterol, and other heart disease risk factors (Menendez et al.).

According to Zawirska-Wojtasiaket al., zucchini is high in manganese, lutein, zeaxanthin, and vitamins A and C, which support good vision and may reduce the risk of age-related eye disorders. It also contains magnesium and vitamin K, which can all help build stronger bones (El-Sayeda). Zucchini is a low-calorie vegetable that is high in water content, which keeps the body from becoming dehydrated, and rich in antioxidants, minerals, fiber, vitamins, especially potassium and vitamin C (Tejada et al.). Because zucchini is low in calories and high in fiber and water, it may help suppress appetite and promote feelings of fullness, which could eventually result in weight loss. It has anticancer and antibacterial properties (El-Sayed, Abiaka). Test-tube and animal research suggest that zucchini extracts have anti-proliferative and pro-apoptotic properties, which may aid in the death or inhibition of the growth of specific cancer cells. Prostatic hyperplasia, an expansion of the prostate that frequently causes urinary and sexual troubles in older men, may be limited by the use of extracts from zucchini seeds, according to studies conducted on animals. Extracted peels from zucchini may help maintain normal thyroid hormone levels, according to rat tests. Eat raw or cooked, zucchini works well in salads, sandwiches, baked dishes, soups, and stews. Because of these nutritional benefits, the attempts have been made in this work to reveal the nutritional composition in raw and cooked extracts.

EXPERIMENTAL METHODS

Chemicals: Analytical-grade general chemicals and solvents were obtained from RANKEM. They are Bovine serum albumin, sodium acetate, EGTA, EDTA, phenonthroline, calcium chloride, magnesium chloride, sodium phosphate, Folin Ciocalteau reagent, ammonium sulphate.

Preparation of extract: About 1Kg of fresh **Zucchini (Summer squash) Classic green** variety was obtained from the super market. 500 g of zucchini were ground with 100 mL of pH 4.5 sodium acetate buffer, then filtered. A 175 mL filtrate was collected, and the leftover material was disposed away. Likewise, 500 g of the material were steam-cooked for 10 minutes at 100 ^{o}C to provide 175 mL of extract. After the extracted material was collected, it was frozen and used for additional analysis.

1. Estimation of Zinc by EDTA titration:

The extract sample was measured out to be about 5 mL, and it was pipetted into a sterile conical flask. added three drops of xylenol orange indicator after being diluted with ten milliliters of purified water. The resultant yellow solution is stirred vigorously with a spatula containing powdered hexamine until it takes on a deep crimson hue. The normal EDTA solution was used to titrate the solution until there was a noticeable change in color from red to yellow. The relationship 1mL of 1M EDTA = 65.38 mg of zinc was used to calculate the quantity of zinc contained in the extract sample.

2. Determination of Manganese using Eriochrome Black –T as an Indicator by complexometric titration:

The concentration of manganese ions (Mn^{2+}) in a sample can be measured quantitatively using complexometric direct titration at pH 10 with EDTA. Eriochrome Black - T is an indication that is wine red when it is complexed with zinc and blue when it is free (HIn^{2-}). Similar to all species that include manganese(II), it easily oxidizes in alkaline environments to produce compounds with an unknown stoichiometry. Manganese(II) is therefore always titrated in the presence of a reducing agent, such as hydroxyl ammonium chloride or ascorbic acid.

$Mn^{2+} + HIn^{2-} \leftrightarrow MnIn^{-} + H^{+}$(pH 10)

$MnIn^{-} + HY^{3-} \leftrightarrow MnY^{2-} + HIn^{2-}$ (pH 10)

A clean conical flask containing 0.5 g of hydroxyl ammonium chloride was pipetted with about 5 mL of the sample extract solution (to prevent oxidation). Warm and diluted to 100 milliliters using boiling distilled water (use a diluted sodium hydroxide solution to neutralize the solution if it's acidic). To keep the manganese in solution, around 3 mL of triethanol amine is added. Next, 2 mL of buffer solution (pH-10) and several drops of EBT indicator are added to turn the mixture alkaline. Titrated with conventional 0.01M EDTA until the color turns blue. It was determined how much manganese was in the sample extract by applying the formula 1mL of 1M EDTA = 27.47 milligrams of Mn.

3. Determination of Iron (III) by ammonium thiocyanate solution by spectrophotometry method:

Iron (III) determination using spectrophotometry and an ammonium thiocyanate solution: When ferric ion and thiocyanate combine, a number of compounds with a strong red color are produced that stay in the solution. Ferrous ions do not react; instead, they can form as $[Fe(SCN)_6]$ depending on the concentration of thiocyanate in the series.3. where the major color species at high thiocyanate concentration is $[Fe(SCN)_6]^{3-}$ where n=1,2,3……,6.

$$Fe^{3+}+6SCN \rightarrow [Fe(SCN)_6]^{3}.$$

It is mostly $[Fe(SCN)_2]^{+}$ at 0.1M thiocyanate concentration and $[Fe(SCN)_6]$ at very high thiocyanate concentrations.3. A significant excess of thiocyanate should be employed in the spectrophotometric determination as this increases the color's stability and intensity. Because sulphate ions have a tendency to form a complex with ferric ions, silver, nickel, copper, uranium, molybdenum, mercury, zinc, cadmium, and bismuth, H_2SO_4 is not advised. If present, interfering stannous and mercury salt must be changed back to stannic and mercury salt in order to prevent the color from being lost. Since they combine to create a very persistent complex with ferric ions, phosphate, arsenate, fluorides, and oxalate all interfere. The influence of phosphate and arsenates can be lessened by using a concentrated acid. Calibration curve was prepared by pipetting 1, 2, 3, 4 and 5mL of 50ppm solution a series of 25 mL of volumetric flask, so as to get the concentration of solution 2, 4, 6, 8 and 10ppm respectively. Add 2.0 mL of thiocyanate and 3mL HCl in each of the flask. The absorbance of the solution reading was took at 480 nm. A standard curve was plotted with the concentration in ppm on X-axis and absorbance of the solution was recorded and entered in the table.

4. Spectrophotometric determination of phosphorus:

One of the main nutrients needed for healthy growth is phosphorus. It takes part in the creation of crucial chemical compounds, including nucleic acids, phospholipids, ATP, and proteins. In 2006, Mendham et al. Although this method has been very helpful for phosphorus measurement, it is thought to be slightly less sensitive than the molybdate blue method. At 460 nm, the complex's absorbance is measured. where the transmitted color is vivid yellow and the absorbed color is blue.

$H_3PO_4^{2-} + 12(NH_4)Mo_7O_{24} + 51H^+ \rightarrow 7(NH_4)_3PO_4.12MoO_3 + 51NH_4^+ + 36H_2O$

Pipette 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL of phosphate solution into a series of 10 mL volumetric flasks. Add 0.8 mL of ammonium vanadate solution and 1 mL of 2.5M HNO_3 solution to each flask and the solutions were allowed to stand for about 15 min. So that color of complex can be developed. Make up to the solution and stirred well in order to get uniform concentration. Similarly the extract was taken and 0.8 mL of ammonium molybdate and ammonium vanadate and 1 mL of distilled water and absorbance of each solution recorded at 480nm. The concentration of phosphate in extract was determined with the help of calibration curve. A calibration curve was plotted with the concentration in ppm on X-axis and absorbance on Y-axis.

5. Determination of Protein by spectrophotometry:

Due to its great sensitivity and ability to measure levels of protein as low as 20 µg, it is the most widely used method for determining the protein content in cell free extracts. A blue-colored complex is produced when copper sulphate and the peptide bonds in a polypeptide chain react in an alkaline media. Furthermore, the phosphomolybdate and phosphotungstate components of the Folin-Ciocalteau reagent are reduced by the tyrosine and tryptophan residues in proteins, resulting in the formation of bluish products that enhance the sensitivity of this technique. It is important to make sure that no substances that interfere with color development are present during sample preparation, such as EDTA, Tris, Carbohydrates, Thiol Reagents, Phenols, etc.

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 Zucchini sample of about 0.1 and 0.2 mL was taken in the standard flask and 1mL of NaOH was added. After adding 5 mL of an alkaline copper sulfate solution to each flask, let them stand for ten minutes. After adding 0.5 mL of Folin's solution, thoroughly stirring, and waiting 10 minutes, the absorbance at 660 nm was determined. The absorbance vs BSA concentration was displayed on the graph. The amount of protein in the sample was calculated using the standard curve.

6. Estimation of amount of Reducing sugar (glucose) by Bertrand's method:

Boiling a known amount of sugar solution with an excess of alkaline copper hydroxide is the method's procedure. The resulting precipitate, cuprous oxide, is dissolved in a warm ferric alum acid solution. After being reduced to FeSO4, the ferric alum is titrated against KMnO4. $10FeSO_4 + 2KMnO_4 + H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 5Fe_2(SO_4)_3 + 8H_2O$ $Cu_2O + Fe(SO_4)_3 + H_2SO_4 \rightarrow 2CuSO_4 + 2FeSO_4 + H_2O$

10 mL of sample solution was pipetted out and 20 mL of $CuSO_4$ solution, 20 mL of alkaline tartrate, and 15 mL 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 acidic ferric sulphate. The filtrate is titrated against standard $KMnO_4$ solution till the permanent pale pink colour appears. From the titrate value, the amount of sugar (glucose) can be calculated.

RESULTS AND DISCUSSION

Zucchini plays a significant role as a food. It is consumed as both vegetable and fruit, is rich in carbohydrates, protein and is used as an important diet due to high water and fibre content (Silva et al.,). It might provide a number of health advantages, including as better digestion and a decreased risk of heart disease (De Melo). The extract from zucchini has a high to moderate vitamin and mineral content. The amount of dark green leaves in the edible section has a direct bearing on this.

Table 1: Comparision of nutritional value of raw and cooked extract of zucchini

SL NO.	NUTRITION FACTS	RAW ZUCCHINI in mg	COOKED ZUCCHINI in mg	% LOSS IN COOKED ZUCCHINI
1	ZINC	1.01	0.15	85
2	MANGANESE	1.02	0.143	86
3	IRON (III)	0.238	0.119	50
4	PROTEIN	0.529	0.09	83
5	PHOSPHOROUS	27	34.8	00
6	SUGAR	3.18	0.1	97

In the present study, 1 Kg of Zucchini was collected from super market; around 500g of Zucchini was grinded and extract was collected. The extract is used for analysis of zinc, manganese, iron(III), phosphorous, protein, reducing Sugar.

The Zinc and Manganese was determined by complexometric titration. It contains a zinc and manganese 1.01 mg and 1.02 mg/100 g of the sample respectively. While cooked Zucchini contains 0.15 mg and 0.143 mg respectively (table 1). Similar results have been observed by Jamuna and Jyothsna. Hence zinc and manganese content reduced on cooking by 85 and 86 % respectively.

Iron (III) content was found to be in raw zucchini was 0.238 mg, while in cooked zucchini it was about 0.119 mg (table 2). Hence 50% loss has been recorded. Similar results have been reported by Jamuna and Shivaprasad in *Rheum palamatum.*

Phosphorous was analyzed by spectrophotometry and was found to be 27mg, where as in cooked zucchini it was about 34.8mg (table 3). Hence there is no loss of phosphorous after cooking, but the amount has increased. On contrary 82% loss has been reported in the *Rheum palamatum* by Jamuna and Jyothsna.

Protein was analyzed by Lowry's method and was found to be in raw Zucchini contains 0.529 mg/100g, While Cooked Zucchini contains about 0.09 mg/100g (table 4). On cooking

83 % of protein has been lost. In the case of leafy stalk of *Pak Choi* only 10% loss has been observed by Jamuna.
Reducing sugar was determined by Bertrand's method, exhibited a glucose content about 3.18 mg where as cooked Zucchini contain 0.1 mg of glucose. So cooked extract exhibits 97% reduction in glucose. The above results are in agreement with the results obtained by Saikia and Mahanta.

Table 2: Determination of Fe^{3+} in Zucchini

Volume of Fe(III) in mL	Volume of thiocayanate in mL	Volume of HCl in mL	Absorbance
0.4	2	3	0.190
0.8	2	3	0.369
1.2	2	3	0.553
1.6	2	3	0.741
2.0	2	3	0.890
Sample-1	4	3	0.338
Sample-2	4	3	0.769

Table 3: Determination of Phosphorous in Zucchini

Volume of stock solution in mL	Volume of ammonium molybdate in mL	Volume of ammonium vanadate in mL	Volume of 2.5M HNO_3 in mL	Absorbance
0.2	0.8	0.8	1	0.100
0.4	0.8	0.8	1	0.153
0.6	0.8	0.8	1	0.221
0.8	0.8	0.8	1	0.266
1.0	0.8	0.8	1	0.338
1.2	0.8	0.8	1	0.378
Sample 1	0.8	0.8	1	0.192
Sample 2	0.8	0.8	1	0.376

Table 4: Determination of protein in Zucchini by Lowrys et al. method

Volume of BSA solution in mL	0.1N NaOH in mL	$CuSO_4$ in mL	Mix and keep it for 10 minutes	Folin's reagent in mL	Keep it for 30 min	Absorbance
0.05	0.95	5		0.5		0.164
0.1	0.90	5		0.5		0.250
0.15	0.85	5		0.5		0.352
0.20	0.80	5		0.5		0.394
0.25	0.75	5		0.5		0.471
0.30	0.70	5		0.5		0.534
0.35	0.65	5		0.5		0.666
0.40	0.60	5		0.5		0.755
Sample 1	0.70	5		0.5		0.727
Sample 2	0.70	5		0.5		0.324

CONCULSION

In the present investigation the extracts collected from the raw and cooked Zucchini were analyzed for biochemical compositions such as Zinc, Manganese, Iron, Phosphorous, Protein, reducing sugar. It is evident that the raw Zucchini maintains higher levels of certain heat sensitive nutrients, by cooking of Zucchini, reduction of these nutrients was observed. Hence it can be consumed as salads, in soups and slices like cucumber instead of cooking. Like all leafy vegetables, Zucchini contains 17 calories in 100g. Zucchini contains a low calories of fat and cholesterol, hence it is used in cardio vascular diseases. Because of its health benefits present investigation was undertaken to study its biochemical composition. It is rich in chief nutrients like Zinc, Phosphorous. Rich in lutein, zeaxanthin, and vitamin A, zucchini supports good eyesight and eye disorders. Certain cancer cells may be killed off or have their growth inhibited by zucchini extract. Extract from zucchini peels may support stable thyroid hormone levels. Because zucchini is minimal in calories, fat, and cholesterol, it is used to treat cardiovascular disorders. Therefore, the goal of the current work is to examine its biochemical makeup.

List of abbreviations:
FAS – Ferric Ammonium Sulphate
EDTA – Ethylene Diamine Tetra Acetate
BSA – Bovine Serum Albumin
SCN – Thiocyanate
EBT – Eriochrome Black T
Ph – Phenanthrolein
$[Fe(SCN)]^{2+}$ – Ferric thiocyanate
ppm – parts per million
Kg – Kilogram mg – milligram
mL – milliliter

Chemicals Molecular Formulae:
KOH – Potassium hydroxide
Ce (IV) – Cerium IV sulphate
$ZnSO_4.7H_2O$ – Zincsulphate heptahydrate
KSCN – Potassium thiocyanate

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