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Nutritional Enhancement of Pigeon Pea (Cajanus Cajan) Through Sprouting: A Quantitative Study of Ascobic Acid (Vitmin C) and Phosphate Content.

Susan C. Udeh[1], Blessing H Nwazulu[2] and Ngozika S. Ike--Samuel[3].

[1,2]Institute of Public Health, University of Nigeria, Enugu, Nigeria.

[3]Department of Community Medicine, University of Nigeria, Enugu, Nigeria.

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ABSTRACT

Pigeon pea (Cajanus cajan), a leguminous crop, is widely consumed in developing countries for its high nutritional content, particularly its vitamin C and essential micronutrients. Sprouting is known to enhance the nutritional profile of seeds, but there is limited research on its effect on the ascorbic acid (vitamin C) and phosphate content of pigeon pea. Pigeon pea seeds were purchased from Ogbete market in Enugu State, Nigeria, and sprouted over five days. Ascorbic acid content was quantified using an iodine titration method, while phosphate levels were determined via spectrophotometry. Samples were collected and analyzed at 24-hour intervals (Day 0 to Day 5). The ascorbic acid content of pigeon pea seeds showed a increase from 0.088 mg/100g on Day 0 to 0.152 mg/100g on Day 5, with the highest increase observed between Day 3 and Day 5. The phosphate content initially increased from 0.275 mg/100g on Day 0 to 0.718 mg/100g on Day 3, before declining slightly to 0.556 mg/100g by Day 5. Statistical analysis indicated that the changes in both ascorbic acid and phosphate content were significant ($p < 0.05$). The findings suggest that sprouting significantly enhances the nutritional content of pigeon pea seeds, particularly with respect to ascorbic acid and phosphate levels. This increase in nutrient content supports the potential health benefits of incorporating sprouted pigeon pea into diets, especially in regions where micronutrient deficiencies are prevalent.

Introduction

Legumes play a vital role in global agriculture and nutrition due to their high protein content, essential vitamins, and ability to fix nitrogen in the soil, improving soil fertility. Among them, pigeon pea (Cajanus cajan) a perennial member of the family fabaceae which is particularly significant, especially in tropical and subtropical regions. Pigeon pea is a major source of protein for millions of people in Asia, Africa, and Latin America, It is eating directly after cooking and can also be used as major ingredient in biscuit and flavoured snacks (Adeola &Ohizua, 2018; Okpala et al., 2013; Tiwari et al., 2011; Rampersad et al., 2003), also in production of noodles (Yadav et al., 2011). Globally, it ranks as the sixth most important legume, with India being the largest producer and consumer (Varshney et al., 2010). The crop's resilience to drought and its adaptability to marginal soils make it highly valuable in regions with challenging climatic conditions (Ghosh et al, 2023).

Pigeon pea (Cajanus cajan) is a crucial legume that is recognized for its adaptability and role in enhancing food security, particularly in developing countries (Mula and Saxena, 2010). As a subsistence crop, pigeon pea contains high levels of amino acids which includes lysine, tryptophan and methionine, is an essential source of dietary vitamins and minerals particularly vitamins B and is therefore especially important for people living on subsistence diets (Oshodi et al. 2009). However, its nutritional potential is limited by the presence of anti-nutritional factors, such as phytic acid, which reduce the bioavailability of minerals like iron, calcium, and zinc (Dahiya et al., 2013). One promising approach to overcome these limitations is sprouting, a process where seeds are germinated under controlled conditions to enhance their nutrient composition and reduce anti-nutritional factors (Mubarak, 2005). Pigeon pea grows best at a PH between 5.0 — 7.0. It will grow on a wide range soils from coarse to fine textured. It will not do well on water logged days, it has some salt tolerance, it is sensitive to salt spray. FAQ reports that pigeon pea is fairly tolerant of herbicides (Williams and Dale, 1998). It is also good source of thiamin, riboflavin, niacin and grown pigeon pea cultivars ranges between 17.9 and 24.3g/100g (Onyenuga et al, 1995). Pigeon pea consumption is believed to offer health benefits and has long been used in Asia for the prevention and treatment of chronic diseases (Hassan et al., 2016). In Oman, for example, it is commonly used to treat hepatitis, superficial infections, as well as measles and chickenpox (Al-Saeedi & Hossain, 2015). Additionally, pigeon pea has pain-relieving properties and is

Tele:
E-mail address: udehsusanchioma@gmail.com

considered a traditional remedy in India for treating stomatitis and gingivitis (Ganesan, 2008). Pigeon pea is a good source of proteins, carbohydrate and dietary minerals, such as calcium, phosphorus, magnesium, iron, sulphur and potassium. It is also good source of thiamin, riboflavin, niacin and grown pigeon pea cultivars ranges between 17.9 and 24.3g/100g (ICRISAT, 1991).

Table 1. Shows the analytical value of pigeon pea without husk in percent (Singh et al 1990).

Moisture	15.2%
Protein	22.3%
Fat	1.7%
Mineral matter	8.6%
Carbohydrate	57.2%
Calcium	9.1%
Phosphate	0.26%

Pigeon pea is a rich source of carbohydrates, protein, fats, and minerals, playing a key role in the nutrition of humans and animals. Both the mature (dry) and immature seeds of pigeon pea are packed with essential nutrients (Table 2).

Table 2. Nutritional profile of immature and mature pigeon pea seed (US Department of Agriculture, 2019).

Nutritients	Immature Seeds (g/100g)	Mature Seeds (g/100g)
Protein	7.20	21.70
Fat	1.64	1.49
Carbohydrate	23.88	62.78
Thiamine (B_1)	0.40	0.64
Riboflovin(B_2)	0.17	0.19
Niacin (B_3)	2.20	2.96
Vitamin B_6	0.68	0.28
Vitamin C	39.00	-
Vitamin E	0.39	-
Calcium	42.00	130.00
Iron	1.60	5.23
Magnessium	68.00	183.00
Manganese	0.57	1.79
Phosphorous	127.00	367.00
Potassium	552.00	1392.00
Sodium	5.00	17.00
Zinc	1.04	2.76

Spouting is the practice of soaking, drawing and then rising seed at regular intervals until they germinate or sprouts. This can be a semi automated or fully automated process when done on a large scale of commercial use (Chavan 1989).

Sprouting has been widely studied for its ability to improve the digestibility and nutritional quality of legumes. During sprouting, enzymes become activated, leading to the breakdown of complex molecules, which enhances the bioavailability of important nutrients, including vitamins and minerals (Luo, 2014). Specifically, sprouting increases the levels of ascorbic acid (vitamin C), a potent antioxidant that aids in immune function and enhances the absorption of iron (Kul Bhushan, 2010). Additionally, sprouting can modify phosphate levels, which are critical for energy metabolism, cellular function, and bone health (Hertzler, 2020).

In Nigeria, pigeon pea is a vital legume, especially in the northern regions, where it is commonly consumed as a traditional food. It is grown for its seeds, which are rich in protein, minerals, and vitamins, and is often used in stews and soups. Nigeria has increasingly recognized the potential of pigeon pea to improve food security, especially in areas prone to drought, due to its hardiness and low input requirements.

This study explores the nutritional enhancement of pigeon pea through sprouting, with a focus on ascorbic acid and phosphate levels. By analyzing how these key nutrients are affected, the research seeks to demonstrate the potential of sprouted pigeon pea as a more nutritionally valuable food source, capable of contributing to improved health outcomes and better nutrient absorption.

Figure 1. The appearance of pigeon pea in different forms.

Source: Adapted from
https://inhabitat.com/diy-how-to-sprout-seeds-and-beans-on-your-kitchen-counter/
https://specialtyproduce.com/produce/Pigeon_Peas_11653.php
https://www.perumarketplace.com/en/beans/Pigeon__Peas__@2@__Gandul__Beans__-__25Kg__-__50Kg/TIERRA__ORGANICA__S.A.C./A

Methodolgy

Study Design

This study uses an experimental and quantitative design aimed at determining the changes in ascorbic acid (Vitamin C) and phosphate content in sprouting pigeon pea seeds over a five-day period.

Materials and Equipment

The following materials and equipment were used in the study:

Glassware and Equipment: Test tubes, pipettes, capillary tubes, measuring cylinders, retort stands, metal spatulas, beakers, glass burettes, foil paper, absorbent cloths, round bottom flasks, plastic funnels, volumetric flasks, ceramic mortars and pestles, weighing balance, mantle heater, electric shaker, distillation column, and spectrophotometer.

Sample Collection

Pigeon Pea Seeds: Seeds of pigeon pea (Cajanus cajan) were sourced from the Ogbete market in Enugu State, Nigeria. The seeds were selected based on their viability, ensuring that they were not infested with weevils and had been harvested during the immediate past planting season.

Control Sprouting Experiment

Preparation:

The seeds were spread evenly on a plastic tray lined with cotton wool.

Anti-fungal Treatment:

A solution of Jik (a sodium hypochlorite solution) and water was prepared in a 1:4 ratio. This solution was sprinkled over the cotton wool to prevent fungal growth during sprouting.

Covering:

The tray was covered with aluminum foil to maintain moisture and create a suitable environment for germination.

Sprouting Duration:

The seeds were allowed to sprout over five days, with samples taken daily for analysis.

Sequential Sampling and Analysis.

The experiment spanned over a five-day period, with samples collected daily to measure changes in protein content.

Quantitative Analysis of ascorbic acid (Vitamin C) in Sample preparation:

Grinding:

10 g of sprouted pigeon pea seeds and 5 g of acid-washed sand were weighed and ground thoroughly using a mortar and pestle.

Extraction:

The ground mixture was added to 100 ml of distilled water in a round bottom flask and shaken for 2 hours using an electric shaker.

Filtration:

The mixture was filtered using filter paper, and the filtrate was collected.

Titration:

5 ml of the filtrate was pipetted into a conical flask, followed by the addition of 2.5 ml of 1 M H_2SO_4 and 1 ml of starch indicator. The solution was then titrated with 0.05 M iodine solution.

Calculation:

$$X = \frac{Tv \times 0.00886 \times 1000 \times \text{vol of extracted Vit C}}{\text{Volume of extracted} \times \text{weight of sample}}$$

Unit = mg/100g

Where:

Titer Value = measured volume from titration

0.00886 = constant

Quantitative Analysis of Phosphate in Sample preparation:

Digestion: 0.2 g of sprouted pigeon pea sample was digested with 20 ml of a mixture of nitric acid and hydrochloric acid in a 3:1 ratio.

Neutralization: The digested sample was neutralized using ammonia solution to a pH of 7.

Sample Preparation:

10 ml of the neutralized solution was transferred to a 50 ml volumetric flask. Subsequently, 4 ml of 10 N H_2SO_4 was added, followed by 6 drops of stannous chloride.

Spectrophotometric Analysis:

The absorbance of the prepared sample was measured at a wavelength of 650 nm using a spectrophotometer.

Calculation:

$$X = \frac{100 x Tv x 0.0014 x 6025}{\text{Weight of sample}}$$

0.0014 = A constant which is liberated by 1ml of O IN H2SO4

6.25 = protein constant.

Statistical Analysis:

Descriptive Statistics: Data on ascorbic acid and phosphate content were analyzed using descriptive statistics to summarize changes across the sprouting period.

Graphical Analysis:

Concentration trends over time were visualized using graphs to illustrate the changes in ascorbic acid and phosphate levels throughout the sprouting process.

Quality Control:

Replicates:

Each sample analysis was conducted in triplicate to ensure reliability and reproducibility of results.

Calibration:

All equipment was calibrated according to the manufacturer's instructions to ensure accurate measurements.

Blanks and Controls: Reagent blanks were included in each set of analyses to correct for any background interference

Result and Discussion

The results of this study demonstrate that sprouting significantly impacts the nutritional profile of pigeon pea (Cajanus cajan). Over the 5-day sprouting period, notable changes were observed in the concentrations of ascorbic acid, protein and phosphate.

Ascorbic Acid (Vitamin C): The result of ascorbic acid in the sprouting seeds from zero to day five

Table 3

Days of Sprouting	Concentration of Ascorbic Acid (Mg/100g)
0 day	0.088mg/100g
1st day	0.092mg/100g
2nd day	0.106mg/100g
3rd day	0.122mg/100g
4th day	0.142mg/100g
5th day	0.152mg/100g

The ascorbic acid content steadily increased from 0.088 mg/100g on day 0 to 0.152 mg/100g on day 5 as the seeds sprouted. This gradual rise in ascorbic acid can be attributed to the metabolic activation and synthesis of vitamin C during the germination process. Sprouting triggers enzymatic activities that enhance the conversion of precursor molecules into ascorbic acid. This finding is consistent with previous reports that sprouting improves the vitamin C content of various legumes and grains (Alvarez-Jubete et al., 2010; Ellong et al., 2015).

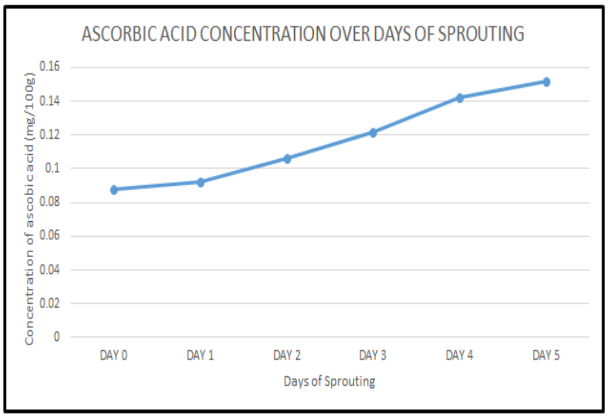

Figure 2. Graph of concentration of Ascorbic Acid against number of days of sprouting

PHOSPHATE

The result of phosphate content in the sprouting of seed of pigeon pea from zero to day five

Table 4

Days of Sprouting	Phosphate Content (Mg/100g)
0 day	0.275mg/100g
1st day	0.70mg/100g
2nd day	1.32mg/100g
3rd day	0.718mg/100g
4th day	0.637mg/100g
5th day	0.556mg/100g

Phosphate content fluctuated throughout the sprouting process. It started at 0.275 mg/100g on day 0, increased to 0.718 mg/100g on day 3, and then decreased to 0.556 mg/100g by day 5. The changes in phosphate levels can be attributed to the dynamic role of phosphorus in various biochemical pathways involved in energy metabolism, cell signalling, and structural components during seed germination and seedling growth (Ae N, et al 1990; ICRISAT, 1991).

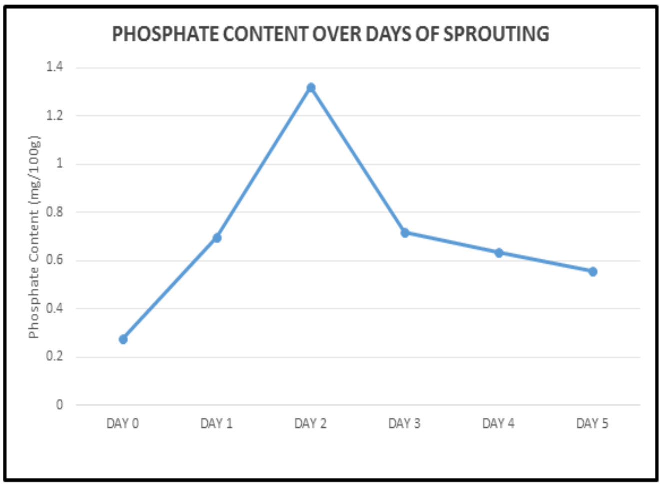

Figure 3. Graph of concentration of Phosphate against numbers of days of sprouting.

The observed changes in the nutritional parameters of pigeon pea during sprouting demonstrate the potential of this simple processing technique to enhance the bioavailability and quality of this important legume crop. The increases in ascorbic acid is particularly significant, as these nutrients play crucial roles in human health and nutrition.

CONCLUSION

In conclusion, this study has shown that sprouting can significantly improve the nutritional profile of pigeon pea (Cajanus cajan). Over the 5-day sprouting period, the concentrations of ascorbic acid increased substantially, while phosphate levels fluctuated. These findings suggest that sprouting can be a useful method to enhance the nutritional value of pigeon pea for food and feed applications. Further research is needed to fully elucidate the underlying biochemical mechanisms responsible for these changes and to optimize the sprouting conditions for maximum nutritional benefits.

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Authors' Contributions

S.U. wrote the first manuscript text and N.I. conducted the analysis. B.N. reviewed the draft manuscript, provided critical comments and suggested additional analyses. S.U. finalized the manuscript which was subsequently reviewed and approved by all authors.

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Data availability

The dataset for the study can be obtained from the corresponding author on request.

DECLARATIONS

Ethical approval and consent to participants.

Not Applicable

Consent for publication

Authors have read and agreed to the submission of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Competing interests

The authors declare no competing interests.

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