

Research Article

Post-harvest Storage-Induced Biochemical Changes in Piper Betle Leaves Cultivated in Kakatpur, Odisha

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Abstract

Piper betle L. is an important ethnobotanical medicine, known for its antiseptic and antimicrobial properties. This study aimed to analyze the phytochemical composition of P. betel leaves from the Kakatapur area in Odisha and to evaluate biochemical changes during storage. Fresh leaves were found to be nutritionally rich, containing proteins (3–3.5%), carbohydrates (0.5–6.1%), crude fiber (2.3%), minerals (2.3–3.3%), and fats (0.4–1.0%). The leaves also contained nicotinic acid, vitamins A and C, thiamine, riboflavin, and enzymes such as diastase and catalase—biochemical analysis of ten different P. Betel varieties showed a decrease in ascorbic acid, nitrogen, and protein levels during storage, while vitamin A and phosphorus levels increased slightly. During storage, leaf aging was characterized by progressive chlorophyll breakdown, leading to white-to-yellow discoloration. Storage in polythene containers with proper drainage and wrapping in banana leaves effectively preserved freshness for up to 21 days. Bleaching began at the base and moved toward the tip after 15 days, often accompanied by soft rot, with bleached leaves displaying a golden-yellow tint. This study provides detailed insights into the phytochemical profile of Indian Piper betel and highlights significant biochemical changes caused by storage, supporting improved post-harvest handling and quality management.

Keywords

Piper Betel, Post-Harvest Storage, Chlorophyll Degradation, Biochemical Changes

1. Introduction

Piper betle, a plant native to India, belongs to the Piperaceae family. It is a popular plant in India. Post-harvest losses in leafy horticultural products remain a major issue because they spoil quickly and undergo rapid metabolic activity [1]. Betel vine, or Piper betle, is an important crop for both the economy and culture in South and Southeast Asia. People value its

leaves not only for traditional chewing but also for their medicinal, antimicrobial, and antioxidant properties, which come from bioactive compounds such as phenolics, flavonoids, and essential oils [2]. However, because betel leaves are very fragile, they are highly susceptible to spoilage after harvest, leading to losses in quality and quantity during storage and transportation. Betel leaves continue to undergo physiological and

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Received: 31 December 2025; **Accepted:** 19 January 2026; **Published:** 17 October 2026



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biochemical changes after harvesting, affecting their shelf life and marketability. These changes are mainly caused by respiration, transpiration, and enzyme activity. As respiration accelerates, stored carbohydrates are used up faster, causing the leaves to lose their freshness and firmness. Simultaneously, increased transpiration results in moisture loss, causing the leaves to wilt and shrink. On a biochemical level, chlorophyll breaks down and turns yellow, while enzymes such as polyphenol oxidase and peroxidase oxidize phenolic compounds, leading to browning and decreased visual appeal. Storage conditions greatly influence these biochemical processes [3]. The rate of metabolic activity in harvested leaves is heavily influenced by factors like temperature, relative humidity, packaging materials, and atmospheric composition. Low-temperature storage generally slows respiration and enzyme activity. However, betel leaves are sensitive to chilling injury, which can damage membranes and accelerate aging under unfavorable conditions. Additionally, modified-atmosphere packaging and suitable coatings have been explored to preserve quality by maintaining moisture and preventing oxidative damage. Besides primary metabolites, secondary metabolites in betel leaves also change considerably during storage [4]. Variations in phenol content, essential oil composition, and antioxidant activity directly impact the leaves' therapeutic and commercial value. To develop effective post-harvest management strategies, understanding the biochemical changes during storage is crucial. This study aims to examine these biochemical changes in betel leaves during storage and identify optimal conditions to minimize degradation while maintaining quality. These insights will help extend the shelf life of this valuable crop, reduce post-harvest losses, and increase profitability.

2. Materials and Methods

2.1. Collection of Plant Leaves

The freshness and health of the Piper betle leaves were confirmed by a trained botanist at Utkal University. We carefully cleaned the selected leaves to remove any dust or dirt that might have clung to them. Then we placed them in a polyethylene container lined and covered with fresh banana leaves. To mimic traditional storage conditions and prevent excess moisture buildup, appropriate arrangements were made for aeration and drainage. The leaves were kept in a room with standard conditions for a set amount of time. Afterward, they were systematically sampled and subjected to biochemical tests to assess changes in their metabolic components and overall quality during storage [5].

2.2. Biochemical Assay

2.2.1. Plant Material and Storage Conditions

We obtained fresh, mature, disease-free Piper betle leaves from the local market and had a botanist at Utkal University

examine them. To remove surface dirt, the leaves were gently washed with distilled water and then air-dried at room temperature. Afterward, the samples were placed in polythene containers lined with fresh banana leaves. This ensured they received adequate air and moisture, similar to normal storage conditions. The leaves were stored in the lab at room temperature ($25 \pm 2^\circ\text{C}$) and relative humidity (60–70%) for a designated period. Biochemical analysis was conducted on samples collected at regular intervals.

2.2.2. Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade. Standard reagents required for phosphorus, chlorophyll, and amino acid estimation were prepared following established protocols.

2.2.3. Estimation of Total Phosphorus & Chlorophyll Content & Free Amino Acids

The molybdenum blue method was used to determine the phosphorus content in the leaf samples. Briefly, a known amount of dried leaf material was digested with a mixture of nitric acid and perchloric acid. The digested sample was then reacted with ammonium molybdate to form a blue complex, which was reduced with an appropriate reducing agent. The colour intensity was measured at 660 nm using a standard calibration curve prepared with known phosphate concentrations, and the phosphorus content was calculated [6].

Chlorophyll content (chlorophyll a, chlorophyll b, and total chlorophyll) was estimated following the method of D. I. Arnon. Fresh leaf tissue was homogenized in 80% acetone and centrifuged to obtain a clear extract. The absorbance of the supernatant was recorded at 645 nm and 663 nm using a UV-Vis spectrophotometer. Chlorophyll concentrations were calculated using standard equations and expressed on a fresh weight basis [7].

Free amino acids were measured using the ninhydrin method. Leaf samples were ground in ethanol and centrifuged to extract the solution. The extract was treated with ninhydrin and heated in a boiling water bath to develop a purple colour. After cooling, the absorbance was read at 570 nm [8]. Glycine served as a standard, and the amino acid content was expressed as mg/g of leaf tissue.

2.2.4. Estimation of Ascorbic Acid & Vitamin A (β -carotene)

Ascorbic acid content was determined using the 2,6-dichlorophenol indophenol dye titration method as described by Roe and Kuether [9]. Leaf extract was prepared in metaphosphoric acid and titrated with a standard dye solution until a faint pink endpoint was reached. Results were expressed as mg ascorbic acid per g fresh weight.

Vitamin A content was estimated as β -carotene after extraction with acetone and petroleum ether. The absorbance was

measured at 450 nm using a spectrophotometer, and the concentration was determined based on standard β -carotene values [10].

2.2.5. Estimation of Total Nitrogen & Crude Protein

Total nitrogen content was determined using the Kjeldahl method. Dried leaf samples were digested with concentrated sulfuric acid in the presence of a catalyst [11]. The digested sample was neutralized, distilled, and titrated to estimate nitrogen content.

Protein content was calculated from total nitrogen by multiplying by a conversion factor (generally 6.25), following standard procedures [12].

2.2.6. Estimation of Total Sugar

Total sugars were estimated by the phenol-sulfuric acid method described by Dubois et al. [13]. Leaf extract was treated with phenol and concentrated sulfuric acid, and absorbance was measured at 490 nm. Glucose was used as a standard.

2.2.7. Estimation of Reducing Sugars

Reducing sugars were determined using the dinitrosalicylic acid (DNS) method. The extract was reacted with the DNS reagent, heated, and the absorbance was recorded at 540 nm. Results were expressed using a glucose standard curve [14].

2.2.8. Estimation of Starch

Starch content was estimated by hydrolyzing the residue left after sugar extraction into glucose, followed by quantification using the anthrone method. Absorbance was measured at 620 nm, and starch content was calculated accordingly [15].

2.2.9. Estimation of Total Phenols

Total phenolic content was determined using the Folin–Ciocalteu method [16]. The leaf extract was reacted with Folin–Ciocalteu reagent and sodium carbonate, and the absorbance was measured at 765 nm. Gallic acid was used as a standard, and results were expressed as mg gallic acid equivalents per g sample.

All analyses were performed in triplicate, and results were expressed as the mean $\pm$ standard deviation. Statistical significance between fresh and stored samples was evaluated using appropriate statistical tools.

3. Results and Discussion

Table 1 shows that the biochemical changes associated with storage in Piper betle leaves are highly significant. The total phosphorus content increased gradually during storage, reaching its highest point in the Ghanghate Bangla variety (263 ppm) on day 21. This increase is probably due to the breakdown of organic compounds and the release of inorganic phosphorus as the leaves age. Meanwhile, the total chlorophyll content was initially high in all leaf samples but decreased steadily over time, indicating that the photosynthetic pigments were breaking down. The leaves of Halisahar Sanchi were completely bleached by day 21, meaning much of their chlorophyll had been lost, making it impossible to measure their chlorophyll levels at that point. Additionally, the total amount of amino acids decreased over time, likely because they are used during respiration and protein breakdown. These results illustrate the biochemical changes that occur during postharvest storage, which affect the quality and shelf life of betel leaves.

Table 1. Changes in total phosphorus (ppm), chlorophyll content, and total amino acid content in leaves of Piper betle varieties during storage over 21 days.

Sl. No.	Varieties	Total phosphorus (ppm)				Total Chlorophyll (mg/gm)				Amino Acid (%)			
		C	7DAS	14DAS	21DAS	C	7DAS	14DAS	21DAS	C	7DAS	14DAS	21DAS
1.	Awani	222	225	231	236	0.95	0.41	0.12	0.05	0.32	0.30	0.28	0.26
2.	Maghi	203	225	229	232	0.68	0.09	0.03	0.27	0.31	0.29	0.28	0.27
3.	Ghanghate Bangla	244	255	258	263	0.47	0.33	0.11	0.06	0.42	0.38	0.36	0.35
4.	Red Stem Bilhari	237	249	252	254	0.52	0.40	0.16	0.02	0.28	0.27	0.25	0.23
5.	Halisahar Sanchi	174	187.5	191.3	200	0.64	0.39	0.78	-	0.41	0.37	0.32	-
6.	Kapuri	151	162.5	169	171	0.37	0.33	0.11	0.02	0.35	0.32	0.30	0.28
7.	Ramtek Bangle	198.2	237.5	243.1	254	0.53	0.33	0.08	0.04	0.39	0.36	0.33	0.31
8.	Gandhi Pan	200	225	236	248	0.71	0.38	0.10	0.05	0.35	0.33	0.30	0.27
9.	Kriele	207	227	232	239	0.83	0.42	0.12	0.06	0.34	0.32	0.29	0.28

Sl. No.	Varieties	Total phosphorus (ppm)				Total Chlorophyll (mg/gm)				Amino Acid (%)			
		C	7DAS	14DAS	21DAS	C	7DAS	14DAS	21DAS	C	7DAS	14DAS	21DAS
10.	Halisahar (W.B.)	175	201	210	212	0.84	0.39	0.08	0.04	0.37	0.34	0.32	0.29

The data in Table 2 show that the ascorbic acid content in Piper betle leaves steadily decreases during storage. Ascorbic acid is an essential antioxidant vital for plant metabolism, especially in maintaining redox balance. It assists in converting quinones into phenols, which helps prevent oxidative browning and protects cells. The reduction in ascorbic acid during storage likely results from its involvement in oxidative reactions and its gradual consumption. The results also reveal that different va-

rieties have significantly different levels of ascorbic acid, indicating genetic variations in their biochemical composition and how they react to storage. In contrast, vitamin A (β -carotene) levels increase during storage. This rise could be due to the relative concentration effect caused by moisture loss or biochemical changes that promote carotenoid accumulation. These distinct patterns of antioxidant components demonstrate that betel leaves' metabolism undergoes complex changes during post-harvest storage, affecting their nutritional and market value [17].

Table 2. Changes in ascorbic acid and vitamin A (β -carotene) levels in the leaves of Piper betle during storage for over 21 days.

Sl. No.	Varieties	Ascorbic acid (mg/100 g)				Vitamin A (mg/100 g)			
		C	7DAS	14DAS	21DAS	C	7DAS	14DAS	21DAS
1.	Awani	177	167	163	160	255	585	612	650
2.	Maghi	182	164	159	152	330	415	486	676
3.	Ghanghate Bangla	183	173	168	162	265	280	410	520
4.	Red Stem Bilhari	195	190	187	181	245	365	418	490
5.	Halisahar Sanchi	183	181	180	-	340	470	590	-
6.	Kapuri	178	168	163	161	320	510	600	612
7.	Ramtek Bangle	207	200	189	185	340	430	512	665
8.	Gandhi Pan	200	190.2	187	174	310	495	601	650
9.	Kriele	183	181	178	176	320	485	492	498
10.	Halisahar (W.B.)	238	194	186	178	295	575	680	720

C: control; DAS: Days after storage

Table 3. Changes in Nitrogen and Protein in the Leaves of Piper betle during Storage over 21 days.

Sl. No.	Varieties	Nitrogen (%)				Protein (%)			
		C	7DAS	14DAS	21DAS	C	7DAS	14DAS	21DAS
1.	Awani	0.41	0.35	0.32	0.24	2.58	2.18	2.00	1.5
2.	Maghi	0.34	0.31	0.28	0.2	2.00	1.93	1.75	1.25
3.	Ghanghate Bangla	0.68	0.34	0.31	0.26	4.25	2.15	1.93	1.62
4.	Red Stem Bilhari	0.56	0.51	0.46	0.32	3.54	3.18	2.87	2.00
5.	Halisahar Sanchi	0.34	0.32	0.3	-	2.12	2.00	1.87	-

Sl. No.	Varieties	Nitrogen (%)				Protein (%)			
		C	7DAS	14DAS	21DAS	C	7DAS	14DAS	21DAS
6.	Kapuri	0.50	0.42	0.38	0.3	3.16	2.62	2.37	1.87
7.	Ramtek Bangle	0.38	0.22	0.18	0.14	2.38	1.37	1.12	0.87
8.	Gandhi Pan	0.32	0.28	0.21	0.14	2.00	1.75	1.31	0.87
9.	Kriele	0.46	0.22	0.18	0.1	2.91	1.37	1.12	0.62
10.	Halisahar (W.B.)	0.27	0.24	0.2	0.16	1.70	1.5	1.25	1.00

Table 3 indicates that the nitrogen content in different types of Piper betle leaves gradually decreases during storage. This reduction in nitrogen levels is linked to the ongoing breakdown of nitrogenous compounds after harvest. A corresponding decrease in protein content is also observed, indicating rapid protein breakdown during storage. This proteolysis may

be related to increased enzyme activity and higher respiration rates, which convert proteins into simpler compounds, including amino acids and other nitrogen-containing metabolites [18]. These findings clearly demonstrate that storage significantly impacts the nitrogen metabolism of betel leaves, thereby affecting their nutritional quality and shelf life.

Table 4. Changes in total sugar and reducing sugar in the Leaves of Piper betle during storage over 21 days.

Sl. No.	Varieties	Total Sugar (mg/100 g)				Reducing sugar (mg/100 g)			
		C	7DAS	14DAS	21DAS	C	7DAS	14DAS	21DAS
1.	Awani	0.35	0.34	0.31	0.23	0.15	0.17	0.08	0.03
2.	Maghi	0.35	0.34	0.33	0.18	0.12	0.23	0.28	0.32
3.	Ghanghate Bangla	0.36	0.35	0.32	0.12	0.11	0.28	0.32	0.36
4.	Red Stem Bilhari	0.30	0.29	0.26	0.11	0.06	0.19	0.22	0.26
5.	Halisahar Sanchi	0.39	0.37	0.08	-	0.06	0.19	0.21	0.22
6.	Kapuri	0.35	0.31	0.29	0.11	0.11	0.21	0.27	0.28
7.	Ramtek Bangle	0.33	0.32	0.31	0.10	0.15	0.25	0.26	0.27
8.	Gandhi Pan	0.36	0.44	0.35	0.14	0.11	0.24	0.34	0.37
9.	Kriele	0.41	0.48	0.43	0.40	0.13	0.33	0.38	0.42
10.	Halisahar (W.B.)	0.36	0.39	0.32	0.11	0.06	0.27	0.31	0.32

The results presented in Table 4 demonstrate that the total sugar content in Piper betle leaves gradually decreased during storage, whereas the reducing sugar content consistently increased over the same period. The decline in total sugars may be attributed to their utilization in respiratory metabolism, which intensifies during post-harvest storage. In contrast, the increase in reducing sugars can be explained by the hydrolysis of complex carbohydrates, particularly starch, into simpler sugar forms such as glucose and fructose. This enzymatic conversion is commonly associated with amylolytic enzymes during senescence [19]. These findings indicate that carbohydrate

metabolism is significantly altered during storage, ultimately affecting the taste, quality, and shelf life of betel leaves.

The observations shown in Table 5 indicate that the starch content in Piper betle leaves gradually decreased during storage. This decrease can be linked to the hydrolysis of starch into simpler sugars during aging, which then contributes to the increase in reducing sugars as previously noted. At the same time, the total phenolic content declined. This reduction may result from the oxidation and enzymatic breakdown of phenolic compounds, primarily mediated by enzymes such as polyphenol oxidase and peroxidase. These biochemical changes reflect the

progression of aging and oxidative processes during storage, ul-

timately causing a decline in the nutritional and functional quality of betel leaves [20].

Table 5. Changes in total sugar and reducing sugar in the Leaves Changes in starch and phenol in the Leaves of Piper betle during Storage over a 21-day period.

Sl. No.	Varieties	Starch (mg/100 g)				Phenol (mg/100 g)			
		C	7DAS	14DAS	21DAS	C	7DAS	14DAS	21DAS
1.	Awani	0.51	0.44	0.33	0.19	211	125	187	180
2.	Maghi	0.65	0.50	0.40	0.23	222	183	155	140
3.	Ghanghate Bangla	0.70	0.36	0.18	0.09	230	190	177	140
4.	Red Stem Bilhari	0.89	0.33	0.21	0.11	222	193	175	150
5.	Halisahar Sanchi	0.60	0.54	0.39	-	177	150	125	115
6.	Kapuri	0.63	0.38	0.23	0.10	201	183	172	165
7.	Ramtek Bangle	0.68	0.54	0.41	0.35	195	183	175	160
8.	Gandhi Pan	0.56	0.55	0.44	0.24	199	187	181	165
9.	Kriele	0.58	0.40	0.31	0.22	177	168	163	158
10.	Halisahar (W.B.)	0.66	0.50	0.36	0.22	124	122	119	90

Therefore, storage induced significant biochemical changes in Piper betle leaves. There was a gradual increase in phosphorus content, whereas chlorophyll, amino acids, nitrogen, protein, total sugars, starch, and phenolic compounds declined during storage. In contrast, the contents of reducing sugars and vitamin A increased, mainly due to the hydrolysis of complex molecules, such as starch, into simpler forms [21]. The decrease in ascorbic acid and phenols indicates enhanced oxidative processes. These collective changes reflect active metabolism and senescence, ultimately leading to deterioration in the nutritional quality and shelf life of betel leaves.

4. Conclusions

The present study emphasizes significant biochemical changes in Piper betle leaves during post-harvest storage. The results clearly show that storage induces dynamic metabolic shifts that ultimately affect the nutritional quality and shelf life of the leaves. An increase in total phosphorus and vitamin A content was observed, likely due to the breakdown of complex biomolecules and concentration effects caused by moisture loss. Conversely, essential biochemical components such as chlorophyll, ascorbic acid, amino acids, nitrogen, protein, total sugars, starch, and phenolic compounds consistently decline as storage time increases. The reduction in chlorophyll content, which causes leaf yellowing and bleaching, indicates the breakdown of photosynthetic pigments, while decreases in ascorbic acid and phenols suggest increased oxidative stress

and diminished antioxidant capacity. The decline in nitrogen and protein levels points to active proteolysis and the utilization of nitrogenous compounds during storage. Similarly, the decrease in total sugars and starch, along with an increase in reducing sugars, confirms enzymatic hydrolysis of complex carbohydrates during senescence. These biochemical changes are mainly driven by respiration, enzymatic activity, and oxidative processes, all of which speed up during storage. Differences among various varieties further highlight the influence of genetics on storage behavior and biochemical stability. Post-harvest storage considerably alters the biochemical composition of betel leaves, leading to a decline in quality. Therefore, proper storage conditions, including temperature and humidity control, are essential to reduce these changes and prolong shelf life. The findings provide valuable insights for improving post-harvest management and maintaining the nutritional and commercial value of betel leaves.

Abbreviations

DAS	Days After Storage
ppm	Parts per Million
UV	Ultraviolet
nm	Nanometer
SD	Standard Deviation

Acknowledgments

The authors would like to acknowledge the Department of Biotechnology, GIET University for continuous support and guidance.

Author Contributions

Romali Sonam Samarpita: Conceptualization, Resources

Manoja Das: Data curation, Methodology

Prasanna Kumar Mishra: Formal Analysis, Investigation

Funding

This work is not supported by any external funding.

Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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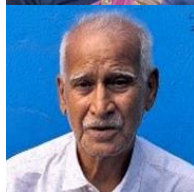
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Biography



Romali Sonam Samarpita is a Ph.D. Research Scholar at GIET University, Department of Botany. She completed her M.Sc. in Botany from Berhampur University in 2017. She is currently doing research on *Piper* family specially *Piper betel* and *Piper nigrum*. Her research field belongs to Biochemistry. She has been doing research at GIET since 2019. She has published a few papers in National Journals and there are two papers under review in International Journals. Also, she has been working as a lecturer in Botany since 2017.



Prasanna Kumar Mishra is an esteemed scholar and former Chemistry Reader at Ravenshaw College. Throughout a dedicated career in higher education, he has contributed significantly to teaching and advancing chemical sciences. Recognized for his solid academic background and mentorship, Dr. Mishra has influenced many generations of chemistry graduates and researchers. His expertise covers both foundational and applied chemistry, with ongoing involvement in academic development and scientific education. As a respected teacher, he is celebrated for his scholarly approach, disciplined teaching style, and fostering scientific curiosity among students. He has authored over 80 research papers and guided 20 PhD students.



Manoj Das is a prominent academic and researcher, currently leading the Department of Biotechnology at Gandhi Institute of Engineering and Technology University, Odisha, Gunupur. With extensive experience in biotech research, teaching, and administration, he has made significant contributions to nanobiotechnology, microbial biotechnology, natural products, and plant biotechnology. His research primarily focuses on green nanoparticle synthesis, probiotics, phytochemical analysis, and the development of bio-functional materials for healthcare and agriculture. Dr. Das has published extensively in reputable national and international journals and mentor students at all levels. He has led numerous projects funded by DBT, BIRAC, MSME, and AICTE.

Research Field

Romali Sonam Samarpita: Botany, Biochemistry

Prasanna Kumar Mishra: Botany, Biochemistry

Manoj Das: Nano Biotechnology, Botany, Plant Biotechnology