

## Full Length Research

# Spectrophotometric and pH Profiling of Biochemical Changes in Intact and Naturally Decayed Onion Tissues (*Allium cepa* L.)

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This study investigated the biochemical differences between intact and naturally decayed onion tissues (*Allium cepa* L.) using spectrophotometric assays and pH profiling. Fresh onion bulbs were purchased from local markets in Sapele, Delta State, Nigeria, and stored under ambient laboratory conditions prior to analysis. Visibly decayed onion tissues were compared with intact tissues to evaluate changes associated with natural spoilage. Hydrolytic enzyme activities (pectinase, cellulase, and protease), DNPH-reactive carbonyl compounds, absorbance at 370 nm, tissue pH, total phenolics, reducing sugars, and soluble protein content were quantified using standard biochemical methods. Decayed tissues showed significantly higher pectinase, cellulase, and protease activities, indicating extensive degradation of structural polysaccharides and proteins. Carbonyl compounds, reducing sugars, tissue alkalinity, and dark pigment index also increased markedly, while total phenolic and soluble protein contents declined substantially. These biochemical alterations suggest enhanced oxidative metabolism, cell wall degradation, and depletion of antioxidant compounds during spoilage. Statistical analysis confirmed significant differences between intact and decayed tissues ( $p < 0.00625$  after Bonferroni correction). The findings demonstrate that integrated spectrophotometric profiling combined with pH measurement provides a rapid, low-cost, and culture-independent approach for assessing onion spoilage. Although the assays used are semi-specific and do not identify particular spoilage organisms, the biochemical patterns observed provide useful indicators of tissue deterioration and may support the development of practical post-harvest monitoring systems, especially in resource-limited settings.

**Keywords:** Onion spoilage; spectrophotometry; pH; pectinase; DNPH-reactive carbonyls; total phenolics; reducing sugars; soluble protein; post-harvest biochemistry

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## INTRODUCTION

Onions (*Allium cepa* L.) are among the most widely cultivated vegetable crops, valued for their nutritional, medicinal, and culinary properties (Slimestad et al., 2007; Sharma et al., 2018). Because onions are harvested seasonally but consumed year-round, large quantities are stored for extended periods under ambient or controlled conditions (Kiran et al., 2024). During storage, they are highly susceptible to spoilage, leading to post-harvest losses estimated at 30–50% under poor storage conditions (Sharma et al., 2018; Suravi et al., 2024). In India alone, post-harvest losses of onions have

been reported at 23–25% of total production, rising to 30–40% in some settings, with rot accounting for the majority of these losses (Sharma et al., 2018). These losses have major economic consequences, especially in developing countries with limited storage infrastructure (Njoroge et al., 2024).

Microbial spoilage typically manifests as soft rot (commonly associated with *Pectobacterium carotovorum*), black mold (*Aspergillus niger*), neck rot (*Botrytis allii*), or *Fusarium* basal rot (Prajapati et al., 2017; Suravi et al., 2024). Most previous studies have focused on identifying the causative microorganisms, with less attention to the biochemical transformations within the onion tissue itself (Kiran et al., 2024). These changes directly influence texture, odor, color, and nutritional quality (Geethanjali et al., 2023). Recent reviews emphasize that a deeper understanding of the biochemical events accompanying spoilage is necessary to develop rapid, non-destructive monitoring systems (Kiran et al., 2024; Mihalev et al., 2023).

During spoilage, plant cell walls are progressively degraded, releasing reducing sugars and other metabolites (Aboaba & Obakpolor, 2000; Ghangaonkar, 2013). In parallel, sulfur compounds undergo alliinase-mediated reactions, producing pyruvate and volatile sulfur compounds responsible for both characteristic onion flavor and the offensive odors of rotten bulbs (Lancaster et al., 1998). Additional metabolic changes include pigment accumulation, pH shifts, phenolic depletion, and protein hydrolysis (Eisenman & Casadevall, 2012; Slimestad et al., 2007). However, many of these biochemical changes have not been systematically quantified in naturally decayed tissue using simple, accessible methods.

Traditional spoilage investigations commonly emphasize microbial isolation and pathogen identification (Suravi et al., 2024). However, biochemical deterioration within the onion tissue itself represents an equally important aspect of post-harvest quality loss (Kiran et al., 2024). The present study therefore adopts a deliberately culture-independent approach centered on chemical and biochemical profiling of visibly decayed onion tissue. While individual biochemical indicators of onion spoilage have been previously investigated, relatively few studies have integrated multiple spectrophotometric indicators and pH into a single biochemical profiling framework for naturally decayed onion tissues under commercial storage conditions. The present work therefore emphasizes integrated biochemical pattern assessment rather than discovery of entirely novel spoilage biomarkers. The objective was not to identify specific spoilage organisms, but rather to characterize measurable biochemical differences associated with naturally occurring tissue deterioration using accessible spectrophotometric assays and pH measurement. Such an approach can provide a foundation for low-cost, rapid quality assessment in storage facilities lacking advanced microbiological infrastructure.

## Methodology

### Sample Collection

Fresh onion bulbs (*Allium cepa* L.) were purchased from local markets in Sapele, Delta State, Nigeria. Following procurement, the samples were transported to the Department of Science Laboratory Technology, Delta State Polytechnic, Otefe-Oghara, where they were stored under ambient laboratory conditions prior to use in the study.

Naturally decayed onion bulbs exhibiting visible signs of spoilage, including tissue softening, discoloration, darkened scales, and tissue collapse, were selected for analysis. Intact onion bulbs showing no visible evidence of deterioration were also selected from the same batch for comparative purposes. Classification of samples was based exclusively on observable physical characteristics of spoilage. Since different visible spoilage conditions were collectively categorized as “decayed,” the biochemical findings should be interpreted as general indicators of onion tissue deterioration rather than evidence of a specific spoilage condition.

No microbial isolation, culturing, staining, or molecular identification procedures were conducted, as the study focused solely on the biochemical profiling of naturally deteriorated onion tissues. Consequently, all biochemical measurements are presented as indicators associated with visibly decayed tissue rather than markers of specific microbial organisms or metabolic pathways.

### Tissue Preparation

Outer decayed scales were removed from spoiled bulbs, and approximately 2 g of decayed mesophyll tissue was excised aseptically from actively decaying regions (lesion margins). For intact onions, 2 g of tissue was taken from middle fleshy scales. Each sample was homogenized in ice-cold 50 mM sodium acetate buffer (pH 5.5, 1:5 w/v) using a chilled mortar and pestle. Homogenates were filtered through double-layered muslin and centrifuged at 10,000 × g for 15 min at 4 °C. Supernatants were stored at –20 °C as crude enzyme extracts. Fresh homogenates (without centrifugation) were prepared separately for pH and soluble metabolite analyses.

## Replication and Statistical Design

For each treatment group (intact and naturally decayed onion tissues), fifteen independent biological replicates were prepared ( $n = 15$  per group). Each biological replicate was analyzed in technical triplicate for all biochemical assays. The mean value of the three technical replicates was calculated and used for subsequent statistical analysis. All results are presented as mean  $\pm$  standard deviation (SD) of the biological replicates.

## Pectinase Activity (DNS Method)

Pectinase activity was determined by measuring reducing sugars released from citrus pectin (Muller et al., 2022). The reaction mixture contained 0.5 mL of 1% (w/v) pectin in 50 mM sodium acetate buffer (pH 5.0) and 0.5 mL crude enzyme extract. After incubation at 40 °C for 30 min, the reaction was stopped with 1.0 mL DNS reagent, boiled for 5 min, cooled, and absorbance read at 540 nm. A glucose standard curve (0–200  $\mu\text{g/mL}$ ) was used ( $R^2 > 0.99$ ). Sample blanks (enzyme extract added after DNS) were subtracted to correct for endogenous reducing compounds. Heat-denatured enzyme controls (extract boiled for 10 min before addition) were run for each biological replicate to confirm that activity was enzymatic. One unit = 1  $\mu\text{mol}$  glucose equivalent released per minute per gram fresh weight. Linear range: 10–150  $\mu\text{g/mL}$ . Detection limit: 2.5  $\mu\text{g/mL}$ .

## Cellulase Activity (DNS Method)

Cellulase activity was measured similarly using 1% carboxymethyl cellulose (CMC) in acetate buffer (pH 5.0). Reaction and detection were as above, including heat-denatured blanks. Glucose standards were used; activity expressed as  $\mu\text{mol}$  glucose equivalent  $\text{min}^{-1} \text{g}^{-1}$  FW.

## Protease Activity (Casein–Folin Method)

Protease activity was determined using casein as substrate (Lowry et al., 1951). Reaction: 0.5 mL 1% casein in 50 mM Tris-HCl (pH 7.5) + 0.5 mL extract, incubated at 37 °C for 30 min. Stopped with 0.5 mL 10% TCA, centrifuged, and supernatant reacted with Folin-Ciocalteu reagent (1:3 dilution). Absorbance at 660 nm. Tyrosine standard curve (0–100  $\mu\text{M}$ ,  $R^2 > 0.99$ ). Blanks: TCA added before incubation (zero-time control) and heat-denatured extract controls. One unit = 1  $\mu\text{mol}$  tyrosine equivalent released  $\text{min}^{-1} \cdot \text{g}^{-1}$  FW.

## Carbonyl Compound Production (DNPH Method)

The assay measures total DNPH-reactive carbonyl compounds directly in tissue extracts, without addition of exogenous substrate. This reflects the pool of  $\alpha$ -keto acids (including pyruvate) and other carbonyls (aldehydes, ketones) formed during spoilage.

Procedure: 0.5 mL of crude tissue extract (same as used for enzymes) was mixed with 0.5 mL of 0.1% DNPH (2,4-dinitrophenylhydrazine) in 2 M HCl. The mixture was incubated at 37 °C for 30 min, then 2 mL of 0.6 M NaOH was added. After 10 min, absorbance was read at 520 nm. A pyruvate standard curve (0–200  $\mu\text{M}$ ,  $R^2 > 0.99$ ) was used, and results are expressed as  $\mu\text{mol}$  pyruvate equivalents  $\text{g}^{-1}$  FW. Sample blanks (extract with NaOH added before DNPH) were subtracted to correct for background absorbance. No exogenous substrate was added, because the objective was to measure endogenous carbonyl compounds accumulated during spoilage. Detection limit: 5  $\mu\text{M}$ .

## Absorbance at 370 nm (Dark Pigment Index)

Tissue supernatant prepared as described for enzyme extraction was diluted with distilled water (1:4 v/v), and the UV-visible spectrum from 350–450 nm was recorded. Absorbance at 370 nm was recorded. Results are reported as absorbance units (AU). This measurement is referred to as "absorbance at 370 nm (dark pigment index)" and is interpreted as a semi-quantitative indicator of accumulated dark-colored compounds (which may include oxidized phenolics, sulfur-containing pigments, or fungal melanin-like substances) but is not chemically specific.

### pH Measurement

Fresh tissue (1 g) was blended with 5 mL distilled water, filtered, and pH measured potentiometrically with a calibrated meter (standards pH 4.0 and 7.0).

### Total Phenolic Content (Folin-Ciocalteu Method)

Phenolics were extracted with 80% methanol (1:5 w/v). After centrifugation, 0.2 mL extract was mixed with 1.0 mL diluted Folin-Ciocalteu reagent (1:10) and 0.8 mL 7.5% Na<sub>2</sub>CO<sub>3</sub>. Incubated 30 min, absorbance at 765 nm. Gallic acid standard curve (0–200 mg/L, R<sup>2</sup> > 0.99). Results expressed as mg GAE 100 g<sup>-1</sup> FW.

### Reducing Sugar Content (DNS Method)

Tissue extract (0.5 mL) was mixed with 1.0 mL DNS reagent, boiled 5 min, cooled, and absorbance read at 540 nm. Glucose standards (0–200 µg/mL) were used. Results as mg glucose equivalent g<sup>-1</sup> FW. Blanks with buffer instead of extract were included.

### Soluble Protein Content (Lowry Method)

Lowry assay (Lowry et al., 1951): extract reacted with alkaline copper reagent, then Folin-Ciocalteu reagent. Absorbance at 750 nm. BSA standards (0–200 µg/mL). Results as mg protein g<sup>-1</sup> FW

### Statistical Analysis

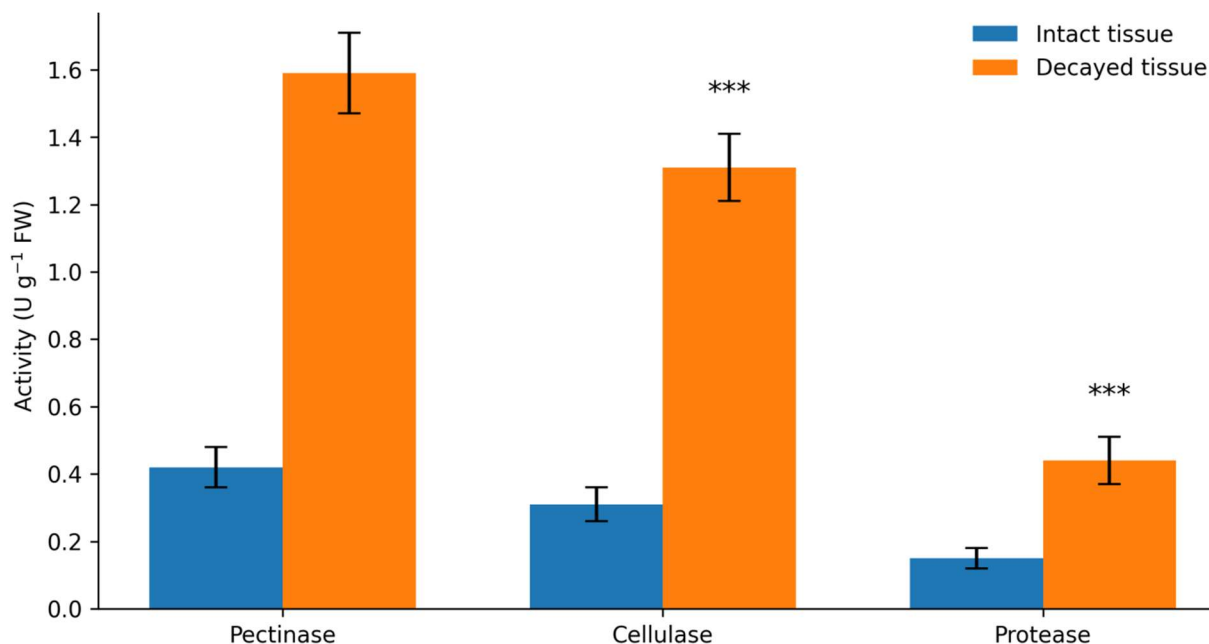
All data were expressed as mean ± standard deviation (SD) from fifteen biological replicates (n = 15), with technical triplicates averaged for analysis. Normality and variance homogeneity were assessed using Shapiro–Wilk and Levene's tests, respectively. Comparisons between intact and decayed onion tissues were performed using a two-tailed Student's *t*-test with Bonferroni correction ( $\alpha = 0.00625$ ). Effect sizes were determined using Cohen's *d*, and analyses were conducted using SPSS version 25.0.

## RESULTS

### Hydrolytic Enzyme Activities

**Table 1. Hydrolytic Enzyme Activities in Intact and Naturally Decayed Onion Tissue, n = 15**

Enzyme parameter	Intact tissue Mean ± SD	Decayed tissue Mean ± SD	Fold change	t(df=28)	p-value	Cohen's d
Pectinase activity	0.42 ± 0.06	1.59 ± 0.12	3.8-fold increase	33.77	<0.001	12.33
Cellulase activity	0.31 ± 0.05	1.31 ± 0.10	4.2-fold increase	34.64	<0.001	12.65
Protease activity	0.15 ± 0.03	0.44 ± 0.07	2.9-fold increase	14.75	<0.001	5.39



Data are mean  $\pm$  SD, n = 15 biological replicates per group. \*\*\* p < 0.001 after Bonferroni correction.

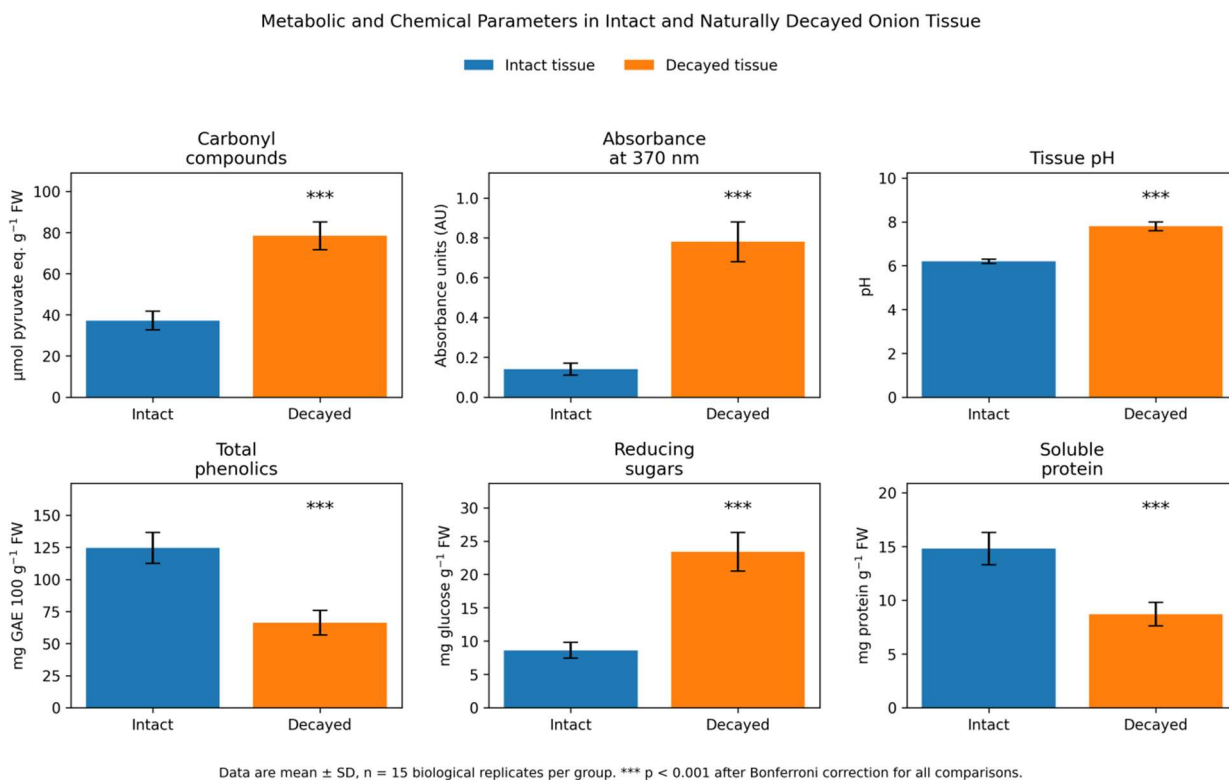
**Figure 1:** Hydrolytic enzyme activities (pectinase, cellulase, protease) in intact and naturally decayed onion tissues.

Data are mean  $\pm$  SD (n=15 biological replicates). \*\*\* p < 0.001, \*\* p < 0.01 (Bonferroni-corrected  $\alpha$  = 0.00625). All comparisons were statistically significant.

## Metabolic and Chemical Alterations

**Table 2:** Metabolic and Chemical Parameters in Intact and Decayed Onion Tissue (n = 15 biological replicates per group)

Biochemical parameter	Intact tissue Mean $\pm$ SD	Decayed tissue Mean $\pm$ SD	Change	t(df=28)	p-value	Cohen's d
DNPH-reactive carbonyls	37.2 $\pm$ 4.5	78.4 $\pm$ 6.8	2.1-fold increase	19.57	<0.001	4.15
Absorbance at 370 nm	0.14 $\pm$ 0.03	0.78 $\pm$ 0.10	5.6-fold increase	23.74	<0.001	5.67
Tissue pH	6.2 $\pm$ 0.1	7.8 $\pm$ 0.2	Alkalinization	27.71	<0.001	5.12
Total phenolics	124.5 $\pm$ 12.1	66.2 $\pm$ 9.5	47% decrease	14.68	<0.001	5.36
Reducing sugars	8.6 $\pm$ 1.2	23.4 $\pm$ 2.9	2.7-fold increase	18.26	<0.001	4.20
Soluble protein	14.8 $\pm$ 1.5	8.7 $\pm$ 1.1	41% decrease	12.70	<0.001	4.64



**Figure 2:** Metabolic and Chemical Parameters in Intact and Naturally Decayed Onion Tissue

Figure 2 shows the key biochemical changes that occurred in onion tissues during natural decay. Compared with intact tissues, decayed onions had noticeably higher levels of DNPH-reactive carbonyl compounds, absorbance at 370 nm, tissue pH, and reducing sugars. These increases suggest intensified oxidative reactions, accumulation of dark-colored compounds, alkalization of the tissue environment, and breakdown of structural carbohydrates during spoilage. On the other hand, total phenolic content and soluble protein levels declined considerably in decayed tissues, indicating loss of antioxidant compounds and ongoing protein degradation. Error bars represent the standard deviation of fifteen biological replicates, and all measured differences were statistically significant after Bonferroni correction ( $p \leq 0.003$ ).

## Discussion

This study provides a quantitative, multi-parameter biochemical profile of naturally decayed onion tissue using only spectrophotometric methods and pH measurement. The results demonstrate that visibly spoiled bulbs exhibit a coordinated set of changes: elevated hydrolytic activities (pectinase, cellulase, protease), accumulation of carbonyl compounds and reducing sugars, increased pH and dark pigment absorbance, and depletion of phenolics and soluble proteins. Importantly, because no microbial isolation was performed, these findings are best interpreted as biochemical correlates of visible spoilage rather than direct evidence of microbial enzyme production or specific pathogen pathways. Nevertheless, the patterns observed are consistent with earlier studies that did identify microorganisms, and they provide a practical basis for rapid spoilage assessment.

## Hydrolytic Enzyme Activities and Cell Wall Degradation

The 3.8 to 4.2-fold increases in pectinase and cellulase activities in decayed tissue align closely with previous reports linking these enzymes to onion softening. Aboaba and Obakpolor (2000) showed that pectinase and cellulase activities were significantly higher in rotten onion bulbs compared to intact ones, and they attributed this to microbial secretion. Similarly, Ghangaonkar (2013) demonstrated that fungi isolated from stored onions produced substantial quantities of hydrolytic enzymes capable of degrading structural polysaccharides. Our study extends these observations by quantifying enzyme activities directly in naturally decayed tissue without prior culturing, thus preserving the endogenous biochemical

context. The concurrent increase in reducing sugars (2.7-fold) supports the interpretation that polysaccharide depolymerization releases soluble sugars, which may serve as substrates for further microbial or enzymatic activity. However, we cannot rule out that some of the measured hydrolytic activity originates from plant endogenous enzymes (e.g., polygalacturonases involved in senescence) rather than exclusively from microorganisms. Future studies using selective inhibitors or gene expression analysis could clarify the relative contributions.

### **Protein Hydrolysis and Soluble Protein Decline**

Protease activity increased 2.9-fold, and soluble protein content decreased by 41%. This pattern is typical of proteolysis during tissue deterioration. Prajapati et al. (2017) reported decreased soluble protein levels in onion bulbs infected with *Aspergillus niger*, and similar findings have been documented in other post-harvest vegetables. Protein breakdown may result from both microbial proteases and plant proteases activated during senescence or stress. The loss of soluble proteins not only reduces nutritional quality but also releases amino acids that can be further metabolized into ammonia and other nitrogenous compounds, potentially contributing to the observed pH increase (discussed below).

### **Carbonyl Compounds and Sulfur Metabolism**

We measured DNPH-reactive carbonyl compounds (expressed as pyruvate equivalents) directly in tissue extracts, without adding exogenous substrate. This approach captures the pool of  $\alpha$ -keto acids and other carbonyls (aldehydes, ketones) that accumulate during spoilage. The 2.1-fold increase in decayed tissue is consistent with enhanced breakdown of S-alk(en)yl cysteine sulfoxides, the characteristic flavor precursors in onions. Lancaster et al. (1998) demonstrated that tissue disruption activates alliinase, leading to rapid production of pyruvate and volatile thiosulfinates. In spoiled tissue, further decomposition of thiosulfinates can generate additional carbonyl compounds. The elevated carbonyl levels may also reflect generalized oxidative stress and lipid peroxidation, which produce aldehydes and ketones. While our method does not distinguish among different carbonyl species, it provides a simple, integrated index of sulfur-related and oxidative metabolism during spoilage.

### **Dark Pigment Accumulation (Absorbance at 370 nm)**

The 5.6-fold increase in absorbance at 370 nm in decayed tissue corresponds with visible dark discoloration (black or brown patches). Several compounds could contribute to this absorbance: fungal melanins (Eisenman & Casadevall, 2012), oxidized phenolic polymers, or sulfur-containing pigments. Salazar-García et al. (2022) used spectrophotometric methods to detect dark pigment-like compounds in the onion pathogen *Sclerotium cepivorum*. However, because the study did not perform chemical confirmation (e.g., solubility in KOH, bleaching with H<sub>2</sub>O<sub>2</sub>, or spectral comparison with authentic melanin), we refer to this measurement as a "dark pigment index" rather than specifically as melanin. The increase in absorbance at 370 nm remains a useful semi-quantitative marker of advanced spoilage, regardless of its exact chemical nature.

### **pH Alkalinization**

Tissue pH rose from 6.2 (slightly acidic) to 7.8 (moderately alkaline) in decayed tissue. This alkalinization is a well-recognized feature of bacterial soft rots, where degradation of organic acids and production of ammonia from amino acid deamination raise the pH (Jiao et al., 2022). In fungal spoilage, pH changes can vary depending on the species and the organic acids produced, but many fungi also secrete ammonia or other basic metabolites. The shift to alkaline pH may further enhance the activity of certain pectinases and proteases that have alkaline optima, creating a feedback loop that accelerates tissue breakdown. The pH measurement is rapid and inexpensive, making it a potentially useful field indicator of spoilage severity.

### **Depletion of Total Phenolics**

Total phenolics decreased by 47% in decayed tissue. Onions are rich in flavonoids (particularly quercetin derivatives)

and other phenolic antioxidants (Slimestad et al., 2007). During spoilage, these compounds may be degraded by microbial enzymes (e.g., polyphenol oxidases, peroxidases) or chemically oxidized. Geethanjali et al. (2023) reported progressive losses of phytonutrients during post-harvest storage of onion varieties. The decline in phenolics not only reduces health-promoting properties but may also compromise the tissue's natural defense against oxidative stress, potentially accelerating further deterioration.

### **Integrated Biochemical Profiling as a Practical Tool**

A major strength of this study is its demonstration that a panel of simple spectrophotometric assays and pH measurement can capture multiple aspects of onion spoilage without the need for microbial culture, molecular diagnostics, or expensive instrumentation. This is particularly relevant for storage facilities in low-resource settings where advanced microbiological infrastructure is unavailable. The assays used here (DNS, Folin-Ciocalteu, Lowry, DNPH, pH meter) require only a basic spectrophotometer, centrifuge, and common reagents. The entire panel can be completed within a few hours, providing a rapid snapshot of spoilage status.

Nevertheless, several analytical limitations must be acknowledged. Spectrophotometric assays are prone to matrix interference from endogenous onion compounds (e.g., sulfur compounds can react with Folin-Ciocalteu reagent). The DNS assay for reducing sugars and enzyme activities may overestimate values if other reducing substances are present. The DNPH method for carbonyls is sensitive but not specific to pyruvate alone. The absorbance at 370 nm is not chemically specific. Therefore, the results should be interpreted as semi-specific biochemical indicators rather than absolute molecular concentrations. Future validation using HPLC or GC-MS on a subset of samples would strengthen the approach.

### **Limitations of the Study**

This study has a few limitations that should be considered when interpreting the results. The research focused on naturally decayed onion tissues and did not involve microbial isolation or molecular identification; therefore, the biochemical changes observed cannot be directly linked to specific spoilage organisms. In addition, different visible forms of spoilage were grouped together under a single “decayed” category, which may have introduced some variation among the samples. The spectrophotometric methods used were also semi-specific and could have been influenced by naturally occurring onion metabolites, such as sulfur compounds and phenolics. As a result, the findings should be viewed as general biochemical indicators of spoilage rather than precise measurements of individual compounds.

### **CONCLUSION**

The study showed that naturally decayed onion tissues experience major biochemical changes that can be detected using simple spectrophotometric methods and pH measurements. Spoiled onion tissues exhibited higher levels of hydrolytic enzyme activity, reducing sugars, carbonyl compounds, pigment accumulation, and alkalinity, while phenolic compounds and soluble proteins decreased significantly. These findings suggest that spoilage involves extensive tissue breakdown, oxidative processes, and a decline in nutritional quality. The study also highlights the value of integrated biochemical profiling as a fast and cost-effective approach for monitoring onion deterioration during storage. Although the methods used cannot identify specific spoilage organisms, the biochemical changes observed provide useful indicators of quality loss and could contribute to the development of practical post-harvest monitoring systems for both research and commercial use.

### **Conflict of Interest**

The authors declare that there are no financial or non-financial conflicts of interest that could have influenced the findings or interpretation of this study. Furthermore, this research did not receive any specific funding from public, private, or commercial funding agencies.

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