

A Rapid, Low-Cost Colorimetric Method for Early Detection of Meat Spoilage Based on the Guaiacol–Hydrogen Peroxide Peroxidase Reaction

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Abstract

Meat spoilage poses significant health risks, especially in resource-limited settings where refrigeration and laboratory testing are unavailable. This study evaluated a simple, low-cost colorimetric method for the early detection of meat spoilage based on the peroxidase-catalyzed oxidation of guaiacol by hydrogen peroxide (H_2O_2). Fresh chicken was stored at ambient temperature (28 °C), and peroxidase activity was assessed over 24 h using an aqueous meat extract mixed with 1% guaiacol and 3% H_2O_2 . Kinetic profiling established a standardized readout time of 2 min. Fresh samples produced a distinct reddish-brown color within this interval, indicating high peroxidase activity. In contrast, the color reaction weakened progressively during storage and became negative (colorless) at approximately 24 h. These findings demonstrate that the loss of peroxidase activity can serve as a rapid, visually readable indicator of meat freshness, offering a practical approach for household-level food safety assessment. Future work should focus on optimizing reagent stability under ambient conditions and developing paper-based test formulations.

Key words

meat spoilage, peroxidase activity, colorimetric detection, food safety, rapid test

1. Introduction

Meat spoilage is a major food safety concern

worldwide, causing foodborne illness and substantial economic losses. Refrigeration and laboratory analysis can control and detect spoilage effectively, but neither

is always accessible. In many rural or resource-limited households, cold storage is unreliable, and analytical instruments are expensive, bulky, and require trained operators, rendering them impractical for everyday use. In such settings, freshness is often judged solely by appearance or odor, which is subjective and unreliable, potentially exposing consumers to unsafe meat. A rapid, low-cost, instrument-free test for meat freshness would therefore be highly valuable.

Peroxidase is an enzyme naturally present in fresh muscle tissue, and its activity declines as meat deteriorates due to denaturation and proteolytic degradation by endogenous enzymes and microbial activity. Consequently, the loss of peroxidase activity has long served as a qualitative indicator of meat freshness and was historically used to identify meat from diseased or deceased animals [1,2]. A convenient method for detecting this activity relies on the guaiacol–hydrogen peroxide reaction. Guaiacol (2-methoxyphenol) is a colorless phenolic compound and a classic chromogenic substrate for peroxidase. In the presence of peroxidase, hydrogen peroxide oxidizes guaiacol into a reddish-brown product, tetraguaiacol, resulting in a distinct visual color change [4,5]. Therefore, a positive reddish-brown reaction indicates active peroxidase and fresh meat, whereas a faint or absent color indicates diminished enzyme activity and potential spoilage.

Conventionally, this reaction is performed in test tubes using meat extracts, which limits portability. Instrumental assays, such as high-performance liquid chromatography, enable precise quantification of peroxidase activity [3]; however, they require specialized equipment and trained operators, rendering them unsuitable for household settings. Although real-time and non-destructive strategies for monitoring meat spoilage have advanced significantly in recent years [5], simple, cost-effective visual tests accessible to consumers remain under-explored.

To address this gap, this study evaluated a dual-reagent colorimetric system based on guaiacol and hydrogen peroxide (H_2O_2) for the early detection of meat spoilage. Specifically, we (i) characterized the color-development kinetics of the reaction to establish an optimal readout time; (ii) tracked changes in peroxidase color intensity in chicken stored at ambient temperature (28 °C) over 24 h; and (iii) monitored the total viable

count (TVC) in parallel to correlate color signal fading with the onset of microbial spoilage.

2. Materials and methods

2.1 Reagents and materials

Guaiacol (1% aqueous solution, AR) was purchased from Macklin Co., Ltd., and hydrogen peroxide solution (3%) was obtained from Hengjian Co., Ltd.; both served as chromogenic reagents. Distilled water was used throughout the study. Fresh chicken breasts were purchased from a local market and tested immediately on the day of acquisition. Other equipment and materials included an analytical balance, a mortar and pestle for tissue homogenization, sealable containers for sample storage, and a digital camera for visual color acquisition.

2.2 Meat samples and storage

Fresh chicken was partitioned into uniform portions and stored in sterile, sealed containers at an ambient temperature of approximately 28 °C. Sampling was conducted at eight time points across a 24 h storage period (0, 0.5, 1, 2, 4, 6, 12, and 24 h) for subsequent analysis.

2.3 Meat extract preparation

At each sampling time point, 1.0 g of chicken tissue was homogenized with 4.0 mL of distilled water using a mortar and pestle. The resulting aqueous meat extract was collected and analyzed immediately.

2.4 Peroxidase colorimetric test

The peroxidase colorimetric assay was conducted in a test tube of meat extract with 0.2 mL of 1% guaiacol solution and 0.1 mL of 3% H_2O_2 . The procedure was adapted from test-tube qualitative protocols described in previous literature [1,2]. A positive reaction was defined as the development of a distinct reddish-brown color, indicating active peroxidase and high meat freshness; a negative reaction was defined as the absence of visible color development, indicating peroxidase inactivation. Chromatic changes were evaluated at a standardized readout time (Section 2.5) and recorded photographically under consistent lighting conditions.

2.5 Color-development kinetics

To establish an optimal readout time, the color-development kinetics of the guaiacol–H₂O₂ oxidation catalyzed by fresh meat extract were monitored continuously over 120 s (at 0, 10, 20, 30, 40, 50, 60, 90, and 120 s). The reaction intensity was quantified using digital image grayscale analysis. The optimal readout window for all subsequent experiments was determined based on the stabilization of color intensity.

2.6 Microbial load assessment

In parallel with the colorimetric assays, the microbial load of the stored chicken was evaluated using commercial TVC test strips according to the manufacturer's instructions. Microbial counts were expressed as colony-forming units per gram (CFU/g). Particular attention was given to identifying the storage time at which TVC reached or exceeded the critical spoilage threshold of approximately 6 log CFU/g [8].

3. Results

3.1 Color-development kinetics of the guaiacol–H₂O₂ reaction

Prior to evaluating stored meat, the color-development kinetics of the guaiacol–H₂O₂ reaction were characterized using fresh chicken extract to establish a standardized readout time. Upon combining guaiacol and H₂O₂ with fresh meat extract, a reddish-brown color developed rapidly, confirming active peroxidase.

As shown in Figure 1, during the 120 s observation window, the color signal (ΔGray) increased rapidly within the first 60–90 s and gradually reached a plateau near 120 s for fresh samples (0–1 h). Conversely, extracts from meat stored for longer durations (4–24 h) exhibited significantly diminished color development slopes and lower maximum ΔGray values, reflecting lower residual peroxidase activity.

Because tetraguaiacol formation is time-dependent, reading the reaction color at a fixed and consistent time point is essential to prevent false-positive or false-negative interpretations. Based on these kinetic profiles, a fixed readout time of 120 s—when color intensity

approaches its plateau—was selected for all subsequent experiments to ensure optimal sensitivity and reproducibility.

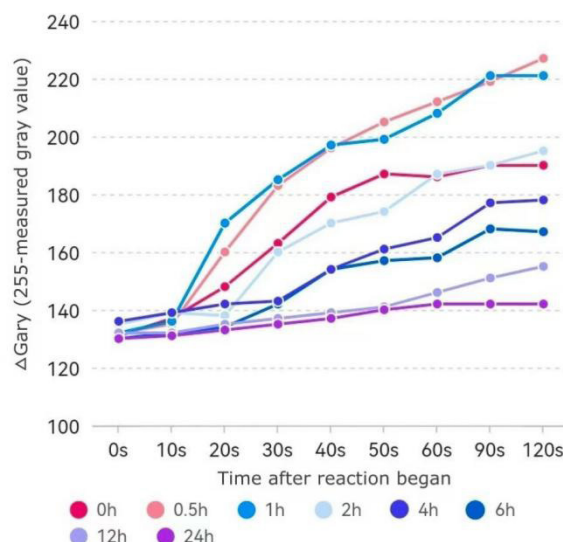


Figure 1: Test solution color changes from 0 to 24 hours

Figure Legend

The y-axis represents the gray value decrease ($\Delta\text{Gray} = 255 - \text{measured gray value}$). An increase in ΔGray over time indicates a progressive darkening of the samples.

3.2 Change in the peroxidase reaction during storage

To track peroxidase activity during spoilage, chicken stored at 28 °C was evaluated at eight sampling intervals over 24 h using the standardized 120 s readout condition. Extracts from fresh chicken (0 h) produced a vibrant reddish-brown color, indicating high enzymatic activity. During storage, the color reaction weakened in a progressive, stepwise manner. Intermediate samples (2–6 h) exhibited a noticeably lighter red tint, and by 12–24 h, the reaction was virtually negative (colorless or light yellow), signifying substantial loss of functional peroxidase (Figure 2).

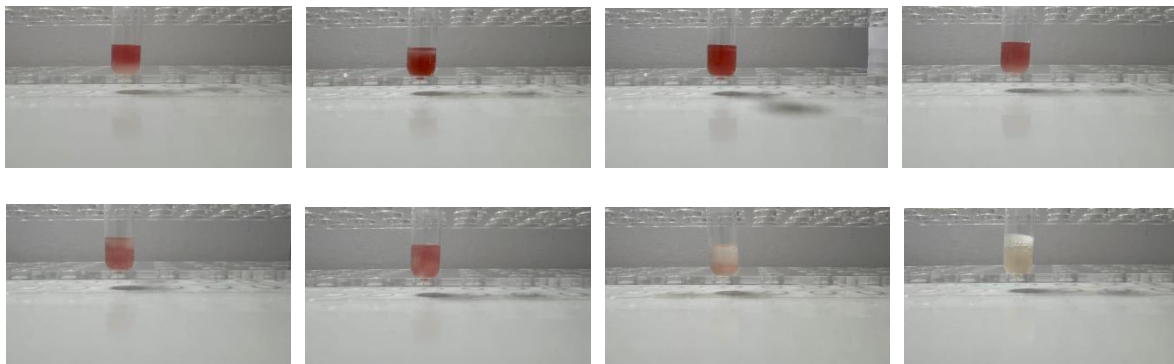


Figure 2: Color of the guaiacol-H₂O₂ test solution for chicken sampled every test during storage at 28 °C (0–24 h).

3.3 Microbial load during storage

Microbial growth was monitored in parallel using total viable count (TVC) test strips. In agreement with sensory spoilage signs (softening, discoloration, and off-odors), TVC values increased steadily over time (Figure 3). The TVC surpassed the threshold commonly recognized as the limit for meat spoilage (6 log CFU/g) between 6 and 12 h. This microbial threshold aligned closely with the storage time at which the peroxidase reaction transitioned from weak (+) to negative (–) (Table 1). This correspondence suggests that the loss of peroxidase activity correlates well with microbial spoilage.

To systematically categorize the color response, reaction intensity was scored on a semi-quantitative four-tier scale:

- Strong (+++): Rapid transition to deep red-dish-brown within 30 s.
- Moderate (++): Clear reddish-brown coloration established within 60 s.
- Weak (+): Pale red coloration developing near 120 s.
- Negative (–): No perceptible color change after 120 s.

As detailed in Table 2, the visual score decreased monotonically from strong (+++) at 0–1 h to negative (–) at 12–24 h. Notably, extracts from 0.5 h and 1 h samples displayed slightly higher Δ Gray values compared to the 0 h sample (Figure 1) [6,7]. This subtle initial surge in

activity may be attributed to early post-mortem cellular changes, where cell membrane disruption increases the solubility and release of bound intracellular peroxidases into the extract, rather than an increase in total enzyme synthesis.

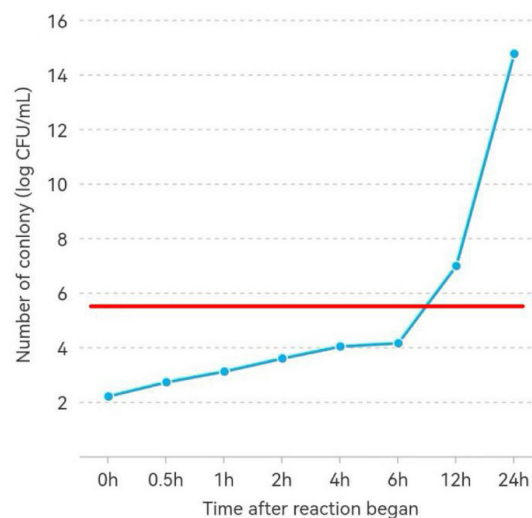


Figure3: Number of colony change from 0 to 24h

The red line is commonly used spoilage level and the figure on the use a log. According to the calculation, 6 log CFU/g has been converted to 5.52 log CFU/mL. This way, the chart becomes more intuitive and allows for a clear visualization of the magnitude of the changes as the value have a huge discrepancy.

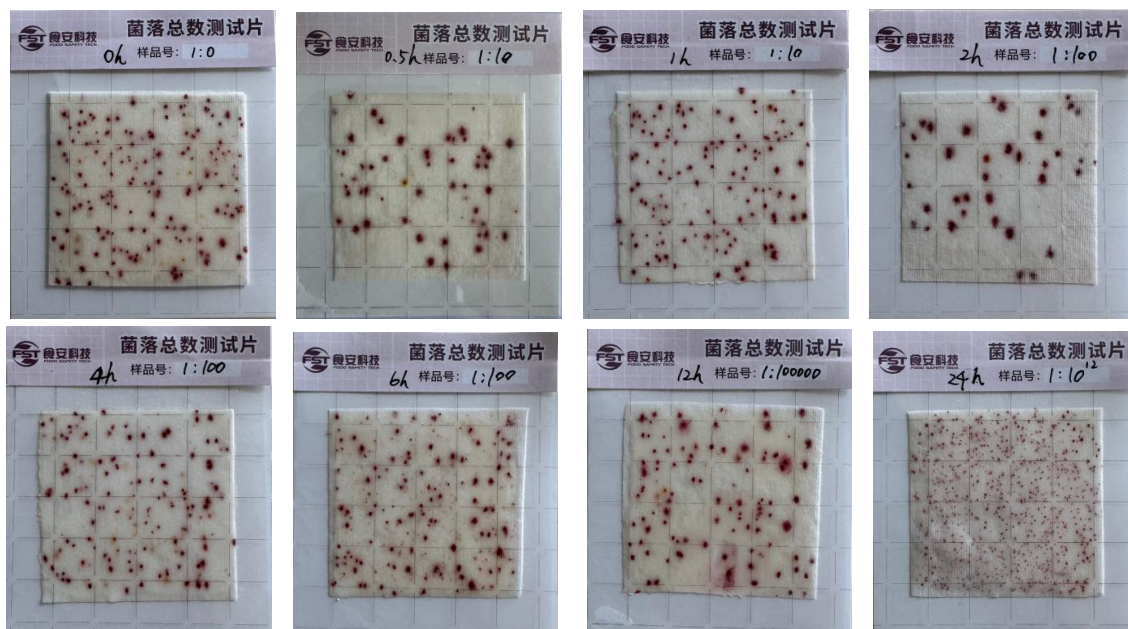


Figure 4: Colony test strip for chicken sampled every test during storage at 28 °C (0–24 h).

Table 1: Dilution rate of the chicken solution used for colony test.

Time(h)	Dilution ratio
0	Undiluted
0.5	1:10
1	1:10
2	1:100
4	1:100
6	1:100
12	1:100000
24	1:10000000000000

Table 2: Visual color score of the guaiacol–H₂O₂ reaction for chicken during storage at 28 °C.

Time (h)	Color score	Interpretation
0	+++	Very fresh
0.5	+++	Very fresh
1	+++	Very fresh
2	++	Fresh
4	++	Fresh
6	+	Not fresh

Time (h)	Color score	Interpretation
12	-	Stale
24	-	Stale

4. Discussion

These results demonstrate that the guaiacol–H₂O₂ colorimetric reaction can effectively distinguish fresh meat from spoiled meat based on peroxidase activity. The progressive weakening of color intensity during storage aligns with a steady loss of enzymatic activity, which can be attributed to enzyme denaturation and proteolytic degradation driven by endogenous enzymes and microbial activity. Notably, the loss of color activity coincided closely with the microbial load surpassing the established spoilage threshold (6 log CFU/g), supporting peroxidase inactivation as a viable biomarker for spoilage onset under ambient conditions.

The proposed method offers several practical advantages. Compared with instrumental techniques such as liquid chromatography [3] or advanced sensors [5], this colorimetric assay requires no electrical equipment, complex instrumentation, or specialized personnel. Unlike traditional sensory evaluations based on odor or appearance—which are highly subjective—this approach provides an objective, visual chemical readout. Furthermore, the minimal sample volume and low reagent costs render it highly suitable for resource-limited households.

Nevertheless, several limitations should be noted. First, the visual assessment remains semi-quantitative, which may introduce ambiguity when evaluating intermediate samples under varying ambient lighting conditions. Second, microbial load was evaluated semi-quantitatively to identify the general onset of spoilage rather than to model exact enzyme inactivation kinetics. Third, the current evaluation was limited to chicken stored under a single ambient condition (28 °C). Finally, the chemical stability of the chromogenic reagents—particularly the light sensitivity of guaiacol—requires optimization prior to practical deployment. Future studies should evaluate diverse meat matrices across multiple storage temperatures and develop dry, paper-based strip formulations to enhance reagent stability and portability.

5. Conclusion

This study demonstrates a rapid, low-cost colorimetric method for the early detection of meat spoilage based on the peroxidase-catalyzed guaiacol–H₂O₂ reaction. The assay reliably differentiated fresh chicken from spoiled samples: the reddish-brown reaction weakened progressively over storage time and completely faded near the point where microbial loads exceeded safety limits. Given its simplicity, speed, and instrument-free operation, this method holds strong potential as an accessible tool for household food safety monitoring, particularly in resource-constrained settings. Further optimization regarding reagent stability and solid-phase test strip integration will facilitate its development into a practical consumer assay.

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