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A comparative analysis of catalase activity in animal and plant tissues collected in Pineville, Rapid Parish, Louisiana, USA

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Abstract

Catalase (EC 1.11.1.6) is a ubiquitous enzyme that catalyzes the disproportionation of hydrogen peroxide (H2O2) into water and oxygen. This study investigated catalase activity in five distinct biological samples: chicken liver, beef muscle, potato tuber, carrot root, and banana pulp. Crude enzyme extracts were prepared from each tissue, and their activity was assessed by measuring the foam height generated upon the addition of a 3% H2O2 solution. The objective was to compare catalase activity across diverse animal and plant tissues, reflecting their varying metabolic demands and roles in oxidative stress management. As hypothesized, chicken liver extract exhibited the highest catalase activity, producing the most vigorous effervescence and greatest foam height (approximately 5–6 cm). Beef muscle demonstrated substantial activity (approximately 3 cm), albeit lower than liver. Potato and banana extracts showed moderate activity (approximately 1.5–2.0 cm), while carrot root extract exhibited minimal activity (<0.5 cm). These findings are consistent with established knowledge regarding catalase distribution, where highly metabolically active and detoxifying organs like the liver contain abundant catalase, and certain plant tissues demonstrate differential levels. The experiment successfully demonstrated that catalase activity varies significantly among different biological tissues, correlating with their specific physiological functions and exposure to reactive oxygen species.

Keywords: Catalase; enzyme; Hydrogen peroxide; Biological samples; Oxidative stress

1. Introduction

Enzymes are macromolecular biological catalysts essential for accelerating biochemical reactions crucial for cellular life. Catalase (EC 1.11.1.6) plays a critical role in cellular defense against oxidative damage by rapidly converting hydrogen peroxide (H2O2), a toxic byproduct of normal aerobic metabolism, into molecular oxygen (O2) and water (H2 O) (LibreTexts, 2018). This reaction is remarkably efficient, with a single catalase molecule capable of transforming approximately 4×107 molecules of H2O2 per second under optimal conditions (LibreTexts, 2018).

Catalase is broadly distributed across oxygen-exposed organisms, from prokaryotes to eukaryotes. However, its concentration and activity vary significantly among different tissues and species, reflecting the organism's specific metabolic requirements and challenges related to oxidative stress, (Chelicani *et al,*2004).. In mammals, the liver is particularly rich in catalase, owing to its central role in detoxification pathways where peroxides and other reactive oxygen species (ROS) are frequently generated and must be neutralized (Giergiel et al., 2015). Muscle tissue also contains catalase, albeit typically at lower concentrations compared to liver. Plant tissues likewise possess catalase to manage H2O2 produced during processes such as respiration and photorespiration (Seis 2015,). The levels of catalase in plants can vary considerably depending on the species, tissue type, developmental stage, and environmental conditions. For instance, some fruits and storage organs exhibit significant catalase activity, while other plant tissues may show very low or negligible activity (Patel & Vora, 2022; Royal Society of Biology, 2019).

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This investigation aimed to compare catalase activity across a diverse set of biological samples: two animal tissues (chicken liver and beef muscle) and three plant tissues (potato tuber, carrot root, and banana pulp). Based on existing literature and physiological roles, it was hypothesized that:

- Chicken liver, being a primary detoxification organ, would exhibit the highest catalase activity.
- Beef muscle would show substantial catalase activity but lower than that of liver.
- Potato tubers and banana pulp would possess measurable, moderate catalase activity due to their metabolic activities and storage functions.
- Carrot roots would exhibit minimal catalase activity, consistent with their primary role in storage and potentially lower metabolic demands related to peroxide production.

The objective of this study was to qualitatively assess and compare the relative catalase activity in these diverse biological samples by measuring the height of the oxygen foam column produced upon exposure of their extracts to hydrogen peroxide. This method provides a clear visual indication of enzymatic H2O2 decomposition, allowing for a comparative analysis of catalase concentrations.

2. Materials and Methods

2.1. Sample Preparation and Enzyme Extraction

Fresh samples of chicken liver, beef muscle (cutlet), potato (*Solanum tuberosum*) tuber, carrot (*Daucus carota*) root, and banana (*Musa acuminata*) pulp were obtained. Approximately 10 g of each tissue was accurately weighed, cut into small pieces, and kept on ice to minimize enzyme degradation.

Enzyme extraction was performed using a cold 0.1 M phosphate buffer (pH 7.0), which provides an optimal pH and temperature for catalase stability. Each tissue sample was individually ground to a fine homogenate using a pre-chilled mortar and pestle with a minimal volume of the cold buffer. The homogenate was then filtered through several layers of cheesecloth to remove large particulate matter. The resulting filtrate was transferred to 15 mL centrifuge tubes and centrifuged at 5,000 × *g* for 15 minutes at 4°C. This centrifugation step pelleted cellular debris, yielding a clear supernatant containing crude catalase enzyme. The supernatant for each sample (liver, muscle, potato, carrot, banana) was carefully decanted into separate labeled tubes and stored on ice until assay to preserve enzyme activity.

2.2. Catalase Activity Assay

Catalase activity was determined by measuring the volume of oxygen gas evolved, which manifests as foam formation, when the enzyme extract was exposed to hydrogen peroxide. All reactions were conducted at room temperature (approximately 22°C), Hossain *et al* 2019

For each sample, 10 mL of the prepared enzyme extract was pipetted into a clean test tube. Subsequently, 2 mL of a 3% (m/v) hydrogen peroxide solution was swiftly added to the extract. Immediately after H2O2 addition, the tube was gently swirled to ensure thorough mixing. The reaction commenced upon contact, with catalase decomposing H2O2 and releasing O2 gas.

The height of the foam column produced in each test tube was used as a semi-quantitative indicator of catalase activity. A ruler was held against the exterior of the test tube, and the maximum foam height, measured from the surface of the liquid to the highest point of the foam, was recorded in centimeters. Measurements were taken approximately 60 seconds after H2O2 addition, allowing the reaction to reach its peak effervescence before foam collapse.

Each sample's activity was assayed in triplicate to ensure reliability and reproducibility of observations. Between trials, test tubes were thoroughly rinsed with distilled water to prevent cross-contamination.

2.3. Controls and Controlled Variables

To ensure that observed bubbling was solely due to enzymatic activity, a negative control was performed. This involved adding 2 mL of 3% H2O2 to 10 mL of plain 0.1 M phosphate buffer (pH 7.0) without any tissue extract. As expected, this control produced no significant foam or bubbling, confirming that H2O2 decomposition in the experimental samples was enzyme-catalyzed.

Several variables were carefully controlled throughout the experiment

- Volume of enzyme extract: 10 mL for all trials.
- Volume of hydrogen peroxide: 2 mL for all trials.
- Concentration of hydrogen peroxide: Consistent 3% solution from the same batch.
- Reaction temperature: All reactions performed at ambient room temperature.
- Timing of measurement: Foam height recorded approximately 60 seconds after H2O2 addition.
- pH of extraction buffer: Maintained at pH 7.0.
- Initial temperature of extracts: Kept on ice until assay.

2.4. Safety Precautions

Gloves and safety goggles were worn at all times when handling hydrogen peroxide. Reactions were performed over a sink to contain any potential spills or overflows.

3. Results

The addition of 3% hydrogen peroxide to the various biological extracts resulted in immediate and visually distinct effervescence, indicating varying levels of catalase activity. Qualitatively, the reactions ranged from highly vigorous, rapid oxygen evolution to minimal or negligible bubbling. Table 1 summarizes the observed foam heights, which served as a semi-quantitative measure of relative catalase activity for each tissue extract.

Table 1 Observed foam heights as an indicator of relative catalase activity in various tissue extracts upon addition of 2 mL of 3% H2O2. Relative catalase activity is visually represented by arrows (more arrows indicate higher activity)

Sample	Observed Foam Height (cm) (Approximate)	Relative Catalase Activity
Chicken liver	5.0–6.0	Very High (⇑⇑⇑)
Beef muscle	3.0	Moderately High (⇑⇑)
Potato (tuber)	1.5–2.0	Moderate (⇑⇑)
Banana (fruit)	1.5–2.0	Moderate (⇑⇑)
Carrot (root)	<0.5	Low (⇑)

The chicken liver extract produced the most pronounced reaction, characterized by immediate, strong effervescence and rapid formation of a stable foam column reaching approximately 5 to 6 cm in height within the 60-second observation period. In some replicates, the reaction was so vigorous that the foam nearly overflowed the test tube, confirming a very high concentration of active catalase.

Beef muscle extract also exhibited significant bubbling and foam formation, albeit less intense than that observed for liver. The foam column in beef muscle trials consistently averaged around 3 cm, suggesting substantial catalase activity, approximately half of that observed in chicken liver under these experimental conditions.

Among the plant extracts, both potato tuber and banana pulp yielded moderate catalase activity. Potato extract produced a steady stream of bubbles that formed a foam column between 1.5 and 2.0 cm. Similarly, banana extract generated a discernible foam column also in the range of 1.5 to 2.0 cm, indicating a positive presence of catalase activity comparable to that of potato.

In stark contrast, the carrot root extract displayed minimal catalase activity. Only a few scattered bubbles were observed, primarily along the sides of the test tube, and no significant foam column developed. The measured foam height was consistently less than 0.5 cm, essentially a thin layer of surface bubbles, suggesting very low or negligible catalase presence in the carrot extract.

Replicate trials for each sample consistently demonstrated the same relative ranking of catalase activity. While slight quantitative variations (approximately ±0.2–0.5 cm) were noted between individual replicates of the same sample, the overall trend remained unequivocal: chicken liver consistently produced the greatest foam height, and carrot root the

least. The buffer-only negative control consistently produced no foam or bubbling, confirming that the observed effervescence in the tissue extracts was solely due to the enzymatic action of catalase.

4. Discussion

The experimental results strongly support the initial hypothesis, demonstrating a clear hierarchy of catalase activity among the tested biological samples: chicken liver > beef muscle > potato ≈ banana > carrot. These findings align well with existing knowledge concerning catalase distribution and its physiological significance in different tissues and organisms.

The exceptional catalase activity observed in chicken liver extract (foam height ≈ 5–6 cm) is consistent with the liver's primary role as a metabolic and detoxification organ. Liver cells (hepatocytes) are abundant in peroxisomes, organelles rich in catalase, which are crucial for decomposing H2O2 generated during various metabolic processes, including fatty acid oxidation and detoxification of xenobiotics (LibreTexts, 2018). Comparative studies have consistently reported liver as one of the tissues with the highest catalase activity in mammals, often significantly exceeding that of other organs (Giergiel et al., 2015). The foam height generated by liver was approximately double that of beef muscle, a difference supported by quantitative studies reporting liver catalase activity to be notably higher than in muscle tissue (Giergiel et al., 2015). This pronounced difference underscores the liver's critical requirement for rapid H2O2 neutralization due to its continuous exposure to metabolic and exogenous peroxides.

Beef muscle extract, while showing substantial catalase activity (foam height ≈ 3 cm), exhibited lower activity than liver. Muscle cells do generate H2O2 as a byproduct of cellular respiration, particularly during intense physical activity, and catalase helps protect against exercise-induced oxidative damage. However, muscle tissue's role in detoxification is secondary to that of the liver, explaining its comparatively lower catalase content. This observation reinforces the principle that catalase levels in a tissue are adapted to its specific physiological function and oxidative load.

The plant tissue results provided insightful comparisons. Both potato tuber and banana pulp extracts yielded moderate catalase activity (foam height ≈ 1.5–2.0 cm). Potatoes, as storage organs, undergo respiratory processes that produce H2O2, and they possess enzymatic systems, including catalase, to manage these reactive oxygen species during storage and sprouting. Bananas, being fruits, also exhibit metabolic activity, particularly during ripening, which can involve the production of ROS. A comparative study of catalase activity in various fruits identified banana as having relatively high catalase activity among tested fruits (Patel & Vora, 2022). Our finding that banana extract produced a definite bubbling reaction, comparable to or slightly less than potato, supports this. While minor variations in foam height between potato and banana could be attributed to experimental precision or biological variability (e.g., ripeness of the banana), both clearly fall into a "moderate catalase" category, indicating significant enzyme presence.

Conversely, the carrot root extract exhibited virtually no catalase activity (foam height $<$0.5 cm). Carrots are primarily storage roots, accumulating carotenes and sugars. Their metabolic activity, particularly concerning H2O2 production, may be lower than that of metabolically active tissues like liver or ripening fruits. Furthermore, plants possess a diverse array of antioxidant mechanisms, including various peroxidase enzymes and non-enzymatic antioxidants, which might be preferentially utilized by certain tissues to manage H2O2 (Royal Society of Biology, 2019). If carrot tissue relies more on peroxidases, which require an electron donor in addition to H2O2 to produce a visible reaction, the simple H2O2 assay would not induce bubbling, thus explaining the lack of foam. The consistent methodology across all plant samples, which successfully extracted active catalase from potato and banana, suggests that the minimal foam in carrot is genuinely indicative of low catalase content rather than an extraction artifact.

It is important to acknowledge the limitations of using foam height as a quantitative measure of enzyme activity. While effective for clear differentiation between samples with high versus low catalase activity, this method is semi-quantitative. Factors such as solution temperature, exact test tube geometry, and the subjective measurement of foam height can introduce variability. For more precise quantitative comparisons, spectrophotometric assays (measuring the disappearance of H2O2 absorbance at 240 nm) or oxygen gas collection methods would be more suitable. Nevertheless, for an educational and comparative study, the foam height method provided a clear, visual, and consistent representation of relative catalase activity, directly proportional to the rate of oxygen gas evolution and thus enzyme activity.

The findings are consistent with existing research on catalase distribution and reinforce the concept that catalase levels in a given tissue are correlated with that tissue's typical exposure to and generation of peroxides. Tissues with high metabolic rates or significant detoxification roles have evolved to contain higher concentrations of catalase (Giergiel et al., 2015). Our observation that some plant tissues like banana exhibit relatively high catalase activity is particularly

interesting, potentially reflecting an adaptation to oxidative stress associated with fruit ripening and other metabolic transitions (Patel & Vora, 2022).

In summary, this experiment successfully confirmed the expected hierarchy of catalase activity: chicken liver > beef muscle > banana ≈ potato > carrot. Each result is biologically plausible given the known physiological roles and metabolic demands of the respective tissues. Minor variations observed were within expected experimental error for a qualitative assay.

5. Conclusion

This laboratory investigation effectively demonstrated significant variations in catalase activity among diverse biological samples, utilizing a straightforward and visually indicative assay based on hydrogen peroxide decomposition. The chicken liver extract exhibited the highest catalase activity, producing vigorous oxygen foam, thereby highlighting the liver's crucial role in detoxifying peroxides. Beef muscle displayed substantial, but comparatively lower, activity, consistent with its physiological demands. Both potato tuber and banana pulp extracts showed moderate catalase activity, confirming the presence of the enzyme in these plant tissues for managing oxidative by-products. In contrast, the carrot root extract demonstrated minimal catalase activity, yielding negligible foam.

These outcomes align with the overarching conclusion that tissues frequently exposed to or generating hydrogen peroxide possess higher concentrations of catalase. The experiment underscored the direct relationship between an organism's metabolic processes and its antioxidant enzyme levels. Furthermore, it provided a clear, visual affirmation of enzyme activity differences and reinforced the understanding that catalase distribution is not uniform but rather physiologically tailored to the specific needs of each tissue.

Future studies could expand upon these findings by employing more quantitative methods, such as spectrophotometric assays, to precisely measure catalase kinetics in each extract. Additionally, investigations could explore how factors like temperature, pH, or substrate concentration influence catalase activity from different sources. Further comparative analysis of catalase in other tissues (e.g., kidney, red blood cells, other fruits or vegetables) or across different species could provide deeper insights into the evolutionary and physiological adaptations of this vital enzyme.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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