Ipomoea batatas (SWEET POTATO) PEEL PEROXIDASE AS AN ALTERNATIVE GLUCOSE OXIDASE- PEROXIDASE FOR SERUM GLUCOSE TEST

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels and often requires regular monitoring to manage the condition and prevent complications. Accurate and accessible glucose testing is therefore essential, particularly in low-resource settings where the cost of commercial diagnostic reagents may be prohibitive. This study explores the potential of sweet potato peel (SPP) as a low-cost, plant-based source of peroxidase for use in glucose diagnostic testing, addressing the demand for accessible reagents in low-resource settings. The main objective was to develop and evaluate a modified glucose oxidase–peroxidase reagent by substituting commercial peroxidase with an enzyme extracted from 300 g of SPP. The peroxidase was partially purified by ammonium sulfate precipitation and dialysis, with protein content and enzymatic activity assessed by the Biuret test and the guaiacol oxidation assay, respectively. The percent yield, specific activity (118 U/mg), and volume required (13.52 mL) to match 4800 units of commercial enzyme were determined. The modified reagent was tested on serum samples from 10 individuals and compared with a standard GOD-POD reagent for accuracy, thermal and pH stability, and turnaround time. Results showed that the SPP-derived reagent performed comparably to the commercial counterpart under optimized conditions (pH 6.0, 2°C), indicating its viability as a substitute. This study demonstrates the potential of SPP peroxidase as a sustainable, cost-effective alternative for glucose testing, with practical implications for improving diagnostic accessibility in underserved communities

Keywords: accuracy, glucose testing, peroxidase, sweet potato peel, sustainability

INTRODUCTION

Background of the Study

Diabetes, a chronic metabolic disorder affecting approximately 422 million people worldwide, has become a major global health concern, particularly in low- and middle-income countries, where 1.5 million deaths are attributed to the disease annually (World Health Organization, 2021). According to a 2014 World Health Organization (WHO) report, 8.5% of adults who were 18 years of age or older had diabetes. By 2019, diabetes had grown to be a major global health issue, contributing to 1.5 million deaths, 48% of which happened in people under 70. Elevated blood glucose levels were associated with approximately 20% of cardiovascular-related deaths, and the condition also contributed to an additional 460,000 deaths due to kidney disease. Standardized diabetes-related mortality rates increased by 3% between 2000 and 2019, with lower-middle-income nations experiencing a 13% increase in diabetes-related mortality (Viana *et al*., 2023). Hyperglycemia, which may occur during fasting or after meals, is a hallmark of diabetes. According to Periasamy *et al*. (2016), the glucose level in diabetic patients ranges between 9 and 40 mM, which is higher than the 3 to 8 mM range observed in healthy individuals. Given the rising prevalence of diabetes, it is imperative to enhance and adapt management and prevention strategies.

The rising burden of diabetes underscores the need for accurate and accessible diagnostic tools. Blood glucose monitoring is essential for effective diabetes management, with

the glucose oxidase method being widely used. This technique measures glucose levels using the enzymes glucose oxidase and peroxidase, typically sourced from *Aspergillus niger* and horseradish, respectively. Horseradish peroxidase (HRP) is the most widely studied and used enzyme due to its high efficiency and versatility in applications such as biosensors and immunodetection (Wang et al., 2024; Akbar et al., 2018). However, HRP production is costly and generates significant waste, prompting research into sustainable alternatives. One promising and affordable option is peroxidase from sweet potato (*Ipomoea batatas*).

Ipomoea batatas (sweet potato) is a widely cultivated and low-cost root crop in many developing countries, including the Philippines. According to data from the Philippine Statistics Authority (2023), the farmgate price of fresh sweet potatoes averaged PHP 20–30 per kilogram, which is approximately $0.35–0.52 USD/kg based on current exchange rates. Notably, the peel, which is usually discarded as waste during food processing, is readily available at no additional cost and may serve as an economically sustainable source of peroxidase.

Despite its availability, there remains a lack of published studies characterizing the peroxidase activity of sweet potato peel specifically for analytical applications such as the glucose oxidase-peroxidase (GOD-POD) method. Although some research has investigated peroxidase activity in sweet potato tubers and other plant sources (Ponce et al., 2004; Mohammadi et al., 2021), the enzymatic efficiency, stability, and practical viability of I. batatas peel-derived peroxidase as a direct substitute for HRP in clinical or diagnostic assays is still underexplored. Therefore, this study aims to address this gap by evaluating the feasibility of using sweet potato peel peroxidase as an alternative enzymatic reagent in the GOD-POD method for serum glucose determination.

The results of this study are expected to benefit two main sectors. For the environment, using SPP helps reduce agricultural waste and promotes sustainable practices by turning a common byproduct into a useful reagent. For the healthcare industry, this low-cost alternative can make glucose testing more affordable and accessible, especially in areas with limited resources. This, in turn, may lead to better disease monitoring and more cost-effective healthcare.

Statement of the Objectives

The study aimed to determine the potential of sweet potato (*Ipomoea batatas*) peel peroxidase as an alternative to the GOD-POD method for serum glucose testing. This study was conducted from January to March 2025.

Specifically, this study sought to achieve the following objectives:

1. To determine the percentage yield of the peroxidase enzyme derived from the initial mass (300g) of the collected SPP.

2. To compare the enzymatic activity of the modified SPP peroxidase reagent with that of the standard GOD-POD glucose reagent in terms of:
   a. Accuracy
   b. Stability
   c. Turnaround time

3. To develop a modified glucose reagent using the extracted SPP peroxidase.

METHODOLOGY

Research Design

The study employed an experimental research design, in which the crude peroxidase extract from sweet potato (Ipomoea batatas) was used as an alternative peroxidase source for glucose determination. The results were compared with the positive control, horseradish peroxidase, to evaluate enzymatic activity, which was measured by spectrophotometry.

Study Site and Sample Collection

Plant specimens were collected from the Solano Public Market located in Solano, Nueva Vizcaya. The laboratory procedures were performed in the research laboratory of Saint Mary's University, which is equipped with the necessary tools and equipment for conducting spectrophotometric assays, peroxidase activity tests, or other relevant experiments.

Specimen Identification

The collected plant specimen was submitted to Nueva Vizcaya State University for certification of its botanical or taxonomic identity.

Blood Donors

Ten college students from Saint Mary's University volunteered to donate blood. Each participant provided signed informed consent as part of the preliminary evaluation process before blood extraction. Donors were instructed to fast for eight (8) hours before blood collection and were advised to avoid taking ascorbic acid during the fasting period. All procedures were conducted under the supervision of a registered medical technologist to ensure compliance with ethical and clinical standards.

Data Gathering Procedure

Collection and Preparation of Plant Sample

Sweet potato peels (SPP) were sourced from Solano Public Market. The tubers were washed, peeled, and the peels were cleaned again to remove contaminants. These prepared peels served as the raw material for peroxidase extraction.

Preparation of Crude Enzyme Extract

A total of 300 grams of SPP was blended with 450 mL of distilled water containing 0.9% NaCl to enhance protein solubility. The resulting slurry was centrifuged at 6000 rpm for 30 minutes. The brownish supernatant was filtered using Whatman No. 1 filter paper and stored at -20°C for enzyme preservation.

Peroxidase Enzyme Assay and Protein Quantification

Enzymatic activity was assessed using a guaiacol-based spectrophotometric assay at 470 nm. The reaction used citrate-phosphate buffer (pH 6.0), guaiacol, H_2O_2, and the enzyme extract. Activity was calculated using the Beer–Lambert Law. Protein concentration was measured using the Biuret test, with BSA as the standard, to assess extract purity.

Purification of Crude Extract and Yield Calculation

Crude extract was purified by ammonium sulfate precipitation (80%), followed by centrifugation and dialysis using CPB (pH 6.0). Enzyme activity and specific activity were

re-evaluated post-purification. Percent yield was computed based on the mass of enzyme obtained versus initial SPP mass.

Formulation of Modified GOD-POD Reagent

To match the 4800 units of activity from commercial peroxidase (30 mg × 160 U/mg), 40.68 mg of purified SPP peroxidase (118 U/mg) was needed. Based on a protein concentration of 3.01 mg/mL, 13.52 mL of the extract was required. This extract was used to replace horseradish peroxidase in the GOD-POD reagent.

Glucose Determination Procedure

Blood samples from 10 adult participants were collected and centrifuged to obtain serum. Glucose concentration was determined using both commercial and modified reagents. The commercial reagent was incubated at 37°C with absorbance read at 600 nm, while the modified reagent was incubated at 2°C and read at 470 nm. A 100 mg/dL glucose standard was used for reference.

Accuracy, Stability, and Processing Time Comparison

Accuracy: Results from both reagents were compared using identical serum samples.
Stability: Enzyme activity was tested under various temperatures (2°C–60°C) and pH levels (4.0–8.0).
Processing Time: Measured from reagent-sample mixing to absorbance reading.

Ethical Considerations

The study was submitted to the University Research Ethics Office (UREO) for ethics review and approval. The office was located on the 2nd Floor, Rev. Fr. John Van Bauwel Hall, SMU Main Campus, Ponce Street, Don Mariano Marcos, Bayombong, 3700 Nueva Vizcaya, Philippines.

RESULTS AND DISCUSSIONS

Section 1. Total Activity, Specific Activity, Protein Content, and Percent Yield

Table 1.

Percent yield of crude and purified enzyme

Sample	Percent yield
Crude enzyme	0.2%
Purified enzyme	0.1%

The percentage yield of the crude peroxidase extract was 0.2%, while the yield of the dialyzed (partially purified) peroxidase extract was slightly lower at 0.1%, highlighting a notable reduction in overall enzyme recovery during purification.

This trend is consistent with the findings of Rathnamsamy *et al*. (2014), who extracted peroxidase from various plant sources and reported that purification steps commonly reduce the percent yield due to the elimination of extraneous proteins and phenolic contaminants. However, they emphasized that even with lower yields, the purified peroxidase exhibited enhanced functionality and was effective in biodegradation applications, highlighting the practical value of purified enzyme despite modest recovery.

Thus, the percent yield shows the balance between efficiency and effectiveness in enzyme extraction and purification, validating the method for potential application in glucose quantification protocols.

Table 2

Enzyme Assay for Crude Enzyme and Purified Enzyme

Time (Seconds)	Absorbance of crude enzyme	Absorbance of purified enzyme
10	1.066	0.612
20	1.714	1.187
30	2.180	1.697
40	2.602	2.092
50	2.921	2.409
60	3.155	2.721
70	**3.301**	**2.959**

Table 2 shows the absorbance values of crude and purified SPP peroxidase measured at 10-second intervals. The absorbance of the crude enzyme ranges from 1.066 to 3.301, while the purified enzyme ranges from 0.612 to 2.959. The data highlights the time-based progression of enzymatic activity for both enzyme preparations. Figure 3 shows the absorbance linearity, and the linear portion was identified from 10 to 70 seconds. The change in absorbance over this period from both crude and purified enzymes was determined. The change in absorbance during this period was used to determine the total peroxidase activity using Beer-Lambert's Law. The total activity for the crude enzyme was 37,810 units, and for the purified enzyme, 35,678 units.

The steady increase in absorbance values for both crude and purified SPP peroxidase reflects active enzymatic function. Liu *et al.* (2019) successfully extracted peroxidase from *I. batatas* stems with high activity, supporting the potential of *I. batatas* parts, including peels, as viable enzyme sources. In a related study, Idesa *et al.* (2018) reported strong peroxidase activity in crude *I. batatas* extracts, which increased with purification, showing a significant difference with the activity of SPP peroxidase. Moreover, a structural analysis of SPP (Li *et al*., 2021), such as that conducted on the 'Zhongshu 1' cultivar, confirms its functional potential in diagnostic assays, reinforcing the feasibility of using SPP peroxidase as an effective, plant-based alternative in GOD-POD systems. These results indicate that both crude and purified enzymes exhibit substantial catalytic activity. Diao *et al.* (2014) demonstrated the application of Beer-Lambert's law to determine enzyme product concentrations, aligning with the approach used in this study. The high total activity observed is consistent with results from similar plant-based enzyme research.

These findings confirm the strong catalytic activity of the crude extract and indicate that sweet potato peel-derived peroxidase may be a suitable alternative to commercial enzymes such as horseradish peroxidase.

Table 3

Biuret Test for Protein Measurement

Extract	Absorbance	Protein in mg
Crude	0.533	**647.54 mg**
Dialyzed	0.248	**301 mg**

Table 3 presents the results of the Biuret test used to measure protein concentration in both crude and dialyzed SPP extracts. The absorbance values for the crude extract and dialyzed extract were 0.533 and 0.248, respectively. Based on the absorbance readings, the protein concentration in the crude extract was calculated to be 647.54 mg, while the dialyzed extract contained 301 mg of protein.

The reduction in protein concentration after dialysis observed in Table 3 aligns with Diao *et al.* (2014), who reported decreased total protein in SPP peroxidase extracts post-dialysis, with retained enzymatic activity. Thermo Fisher Scientific (2024) notes that dialysis effectively removes low-molecular-weight contaminants following ammonium sulfate precipitation. Similarly, Beckman Coulter (2023) highlights dialysis as a key step in enhancing enzyme purity without affecting activity, especially in peroxidase workflows like HRP. These findings support the present study, which showed reduced protein content after dialysis, indicating improved purity rather than enzyme degradation, and confirming dialysis as a reliable purification step for plant-derived peroxidase extracts in analytical applications. The specific activity was calculated using the method described by Diao *et al.* (2014), in which specific activity is defined as the total enzyme activity divided by the amount of protein in the sample. In this study, the crude extract exhibited a specific activity of 58 U/mg, while the purified enzyme demonstrated a markedly higher specific activity of 118 U/mg.

This substantial increase indicates that dialysis effectively removed non-enzymatic proteins and other impurities, thereby enriching the sample with more active peroxidase relative to total protein. Enhancement in specific activity is a strong indicator of improved enzyme purity, as it reflects the proportion of functional enzyme in the protein mixture. These findings reinforce the role of dialysis not only in reducing contaminants but also in concentrating the enzymatically active fraction of the extract.

Section 2. Enzymatic Activity of the Modified and Commercial Glucose Oxidase-Peroxidase Reagents in Different Conditions.

Table 4

Glucose values: Commercial Reagent vs. Modified Reagent

Sample	Commercial reagent	Modified reagent
Control	91 mg/dL	89.88 mg/dL
1	79.71 mg/dL	78.57 mg/dL
2	82.61 mg/dL	82.14 mg/dL
3	71.01 mg/dL	70.24 mg/dL
4	73.91 mg/dL	73.80 mg/dL
5	88.40 mg/dL	86.90 mg/dL
6	71.01 mg/dL	70.23 mg/dL
7	79.71 mg/dL	77.38 mg/dL
8	82.60 mg/dL	82.14 mg/dL
9	71.01 mg/dL	69.04 mg/dL
10	72.46 mg/dL	70.24 mg/dL

Table 4 presents a comparative analysis of glucose concentrations measured using a commercial GOD-POD reagent and a modified reagent containing SPP peroxidase. Ten samples and one control were tested, with glucose levels reported in mg/dL for both reagents. The values obtained with the modified reagent closely matched those of the commercial reagent, differing by only 1 to 2 mg/dL, suggesting potential diagnostic applicability.

This observation supports the potential of SPP peroxidase as a suitable alternative to horseradish peroxidase in glucose quantification assays. In a related study, Centeno *et al.* (2017) successfully developed a source of peroxidase for amperometric glucose bioanalysis using guinea grass. This highlights the potential of plant-based peroxidase to effectively catalyze the hydrogen peroxide produced in bioanalytical assays. Their findings affirm that *I. batatas* peroxidase possesses adequate catalytic potential and compatibility with glucose oxidase, supporting its use as a functional replacement in formulating reagents.

Therefore, the close similarities in glucose readings between the modified and commercial reagents demonstrate the reliability and functional effectiveness of the modified reagent, showing its potential for use in clinical glucose determinations.

Table 5

Peroxidase Exposed to Different pH and Temperature

	Total activity
4	34, 883 U
5	35, 492 U
6	**37, 607 U**
7	35, 796 U
2 °C	**35, 796 U**
24 °C	27, 287 U
37 °C	19, 234 U
60 °C	14, 904 U

Table 5 presents the total enzymatic activity of SPP peroxidase exposed to different pH and temperature conditions. Among the tested pH levels, the highest total activity was observed at pH 6, with a value of 37,607 U, followed by pH 7 (35,796 U) and pH 5 (35,492 U). The lowest activity was observed at pH 8 (19,234 U), indicating a sharp decline in enzymatic efficiency at higher pH values. As for temperature, the difference also significantly affects the total activity of the dialyzed enzyme extract. At 2 °C, the dialyzed enzyme extract exhibited the highest total activity, recording 35,796 U, while the lowest performance was observed at 60 °C, with 14,904 U.

The results align with those reported by Bharadwaj, P.S. (2014), which showed that peroxidase extracted from SPPs exhibited peak activity at pH 6, suggesting that this pH supports the optimal conformation and reactivity of the enzyme's active site. Similarly, a study by Li et al. (2021) on I. batatas peroxidase also identified pH 6–7 as the optimal range for catalytic performance, with a marked decline at pH 8 and above. The reduced enzymatic activity at pH 8 suggests that alkaline environments may denature or distort the enzyme's active site, thus compromising its function. Regarding temperature, the findings also align with previous literature. According to Stănciuc *et al.* (2014), as temperature increases, some enzymes denature, thereby decreasing their activity. A study by Ballesta et al. (2020) also reported that tomato peroxidase remained stable up to 60 °C, but activity decreased significantly at higher temperatures. Specifically, residual enzymatic activity dropped to 60% at 70 °C after 5 minutes of incubation, highlighting the thermal sensitivity of peroxidases above certain temperatures. The comparison between the commercial and modified peroxidase further supports these observations.

These findings suggest that the modified enzyme may be better suited for applications requiring activity in mildly acidic, cold environments, whereas the commercial enzyme performs optimally under neutral, warmer conditions.

Turnaround time

The processing time for both assays was carefully monitored from the start of the reaction until measurable results were obtained. The commercial reagent completed the assay in 12 minutes, demonstrating a relatively rapid turnaround time. In contrast, the modified reagent required 22 minutes to achieve comparable results. This difference in processing time may be attributed to the lower catalytic efficiency of the partially purified enzyme, which prolongs its reactivity.

Section 3. Developed Modified Glucose Oxidase-Peroxidase Reagent

Figure 1.

Modified Glucose Oxidase-Peroxidase Reagent

The image above shows the modified GOD-POD reagent developed in this study. The formulation contains glucose oxidase (GOD), peroxidase extracted from SPP, guaiacol as the chromogenic substrate, and citrate-phosphate buffer (CPB) to maintain optimal pH. This glucose reagent requires specific conditions to function comparably to the commercial reagent, specifically a pH of 6 and an incubation period of 20 minutes at 2 °C.

The modified reagent was designed to evaluate the feasibility of using SPP peroxidase as a substitute for commercially available peroxidase in glucose determination assays. The resulting solution maintained a stable appearance and was used successfully in the enzymatic colorimetric method to detect glucose levels, producing a brownish color upon reaction that indicates the presence of hydrogen peroxide and effective enzymatic activity. However, two possible limitations of the reagent include the reduced enzymatic activity of SPP peroxidase compared to commercial peroxidase, which may reduce sensitivity at low glucose concentrations, and the difficulty in standardizing enzyme concentration, as crude peroxidase extracts may exhibit variability in enzymatic activity depending on the extraction conditions and *I. batatas* variety. These limitations affected the glucose values obtained from the modified reagent. The normal range of the results obtained showed a 1-2 mg/dl difference from the commercial reagent. Despite these limitations, the modified reagent still produced glucose values within an acceptable range, demonstrating its potential as a viable, cost-effective alternative to commercial peroxidase for glucose determination.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

This study evaluated the potential of sweet potato peel peroxidase (SPPP) as an alternative to commercial peroxidase in the glucose oxidase-peroxidase (GOD-POD) method. From an initial 300 g of sweet potato peel (SPP), the percentage yield of the crude peroxidase extract was 0.2% (0.6 g), while the yield after purification through dialysis was 0.1% (0.3 g). This decrease is expected due to the removal of non-enzymatic proteins and other impurities. In terms of enzymatic activity, the modified SPPP reagent was compared with the standard

GOD-POD reagent in terms of accuracy, stability, and turnaround time. The glucose values obtained with the modified reagent showed only a 1–2 mg/dL difference compared with those obtained with the commercial reagent, and this difference was not statistically significant. This slight difference can be attributed to the partial purification of the SPPP. Regarding stability, the modified reagent was most effective at pH 6 and 2 °C, while the commercial reagent was stable at pH 7 and 37 °C. Although the modified reagent had a longer turnaround time of 22 minutes, it remained within acceptable limits for diagnostic use. Overall, a modified glucose reagent incorporating SPPP was successfully developed. The results indicate that with further refinement and purification, SPPP has the potential to be a reliable, cost-effective, and locally sourced alternative to commercial peroxidase in glucose testing.

Recommendations

1. SPP contains various enzymes that may interfere with the peroxidase oxidation process. Further purification of the extract should be done by using more refined techniques, such as multiple-step precipitation or chromatography, to improve the specific activity and reduce enzyme interference in the modified reagent.

2. Assess the shelf-life of the modified SPP reagent to determine how long it remains effective under different storage conditions.

3. Test the sensitivity of the modified reagent by using both hypoglycemic and hyperglycemic samples.

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