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A Portable Paper Strip Biosensor -based Smartphone for Detection of Glucose Content in Rice Samples

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Abstract

A paper strip biosensor was fabricated through the combination of horseradish peroxidase and glucose oxidase with 4-aminoantipyrine and phenol in order to produce a pink color. The color intensity was measured with a smartphone for quantitative analysis. To achieve highest paper strip biosensor performance, the concentrations of glucose oxidase, horseradish peroxidase, phenol, 4-aminoantypyrine, pH, and reaction time were studied. Under optimizations, the linear range of the glucose was 0.5-10 mM, and the detection limit was 0.17 mM. The detection of glucose was achieved within 7 min. The paper strip biosensor was successfully applied to the measurement of glucose in rice samples. The paper strip biosensor exhibits considerable promise as a portable instrument for the detection of glucose in rice and is also applicable to other types of samples. This research, nevertheless, necessitates controlled experimental conditions. Due to the fact that it impacts enzymatic activity.

Keywords: Glucose, Paper Strip, Biosensor, Rice, Smartphone

1. Introduction

The World Health Organization estimates that more than 180 million people worldwide have diabetes and that diabetes-related deaths have impacted approximately 2.9 million people. By 2030, the worldwide prevalence of this disease is anticipated to have doubled (1, 2). A condition characterized by abnormally elevated levels of blood sugar (also known as blood glucose), diabetes is an autoimmune disorder. It is also referred to as hyperglycemia (3). Blood glucose levels are regulated by the dietary intake of nutrients that supply the body's organs, muscles, and neurological system with energy (4). As one of the most extensively consumed foods on a global scale, rice constitutes a substantial component of many diets. It is especially widespread throughout Asia (5, 6).

Rice is primarily comprised of carbohydrates, constituting around 80% of its whole dry weight. The human digestive system is responsible for the breakdown of carbohydrates found in rice into simpler sugar molecules, namely glucose. Subsequently, glucose is absorbed into the circulatory system (7, 8). The glycemic index (GI) can be used to categorize the blood glucose responses elicited by different carbohydrate meals.

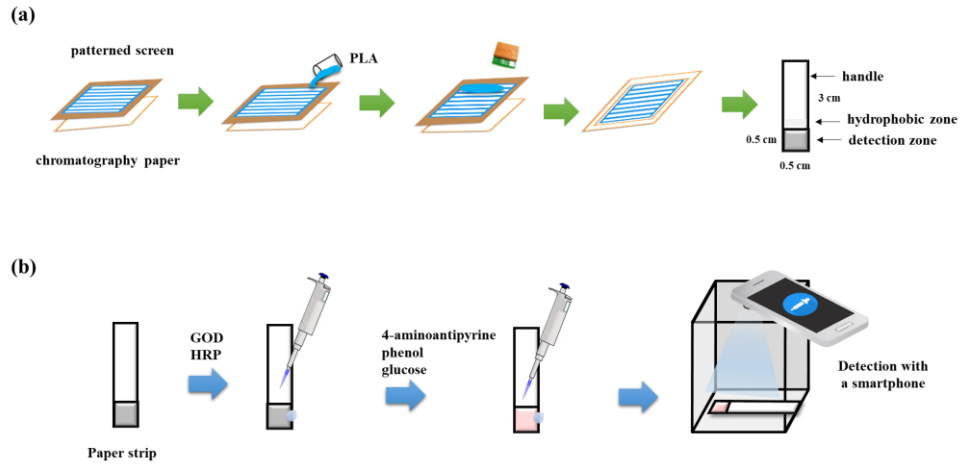

Figure 1 (a) The paper strip production process using the polymer screen-printing technique. (b) Principles of the paper strip biosensor for glucose detection.

Each kind of rice exhibits varying GI levels. The GI is a measure of the blood glucose reaction to the consumption of dietary carbohydrates in food (9, 10). It categorizes these foods into three levels based on their GI values: low (GI < 55), medium (GI: 56–69), and high (GI ≥ 70). Every kind of rice possesses a distinct GI composition. Typically, low GI foods elicit a comparatively lower glucose response compared to high glycemic index foods (11). Therefore, the detection of glucose content in rice is a crucial necessity.

There are numerous methods for detecting and quantifying glucose in food samples available today. These technologies are roughly classified into two types: enzymatic approaches, such as biosensors (12, 13), and non-enzymatic instrumentation, such as high performance liquid chromatography (HPLC) systems and their related detectors (14, 15). The biosensor is based on glucose oxidase (GOD), which can convert glucose to hydrogen peroxide (H2O2) and gluconic acid under aerobic conditions. The chromogenic reaction with indicator can then be accelerated further by horseradish peroxidase (HRP), resulting in a color (16). High selectivity, rapid response times, portability, and compact size are among advantages of biosensors. A colorimetric biosensor utilizing GOD and silver nanoparticles (AgNPs) was developed. The biosensor generates hydrogen peroxide, which in turn causes the AgNPs to disintegrate, resulting in observable changes in color (17). Ghateii and Jahanshahi (18) created colorimetric glucose detection using smartphone-coupled microfluidic paper-based analytical devices (µPADs). The color intensity was generated and subsequently detected using a smartphone.

Here, we fabricate a portable paper strip biosensor for glucose detection in rice using a smartphone as a detector. The fabrication procedure of the portable paper strip biosensor is shown in figure 1. The advantage of this study is that it is easy, involves few steps, and can be measured in the field.

2. Materials and Experiment

2.1 Reagents and materials

All chemicals used in this study were of analytical grade. GOD from Aspergillus niger (EC 1.1.3.4), peroxidase from horseradish (EC 1.11.1.7), and 4-aminoantipyrine were obtained from Sigma-Aldrich (St. Louis, MO, USA). D-Glucose monohydrate was supplied by Ajax Finechem Pty Ltd. (New Zeland). Phenol was purchased from Fisher scientific (UK). Polylactic acid was purchased by Nature Work (Blair, NE, USA). Dichloromethane was purchased from RCI Labscan (Pathumwan, Bangkok, Thailand). Rice samples were collected from Thailand. Patterned screening block was frabricated by Chaiyaboon Brothers Co., Ltd. (Lam Luk Ka, Pathum Thani, Thailand). Whatman chromatography paper No. 4 (25 ×25 cm pure cellulose paper) was obtained from GE Healthcare (Little, Buckinghamshire, UK).

2.2 Apparatus

A smartphone model alcatel shine lite 5080X (Shenzhen, China) was used to take the photograph of a paper test strips and the Color Picker Application was used to measure RGB values. The paper test photograph was recorded by the homemade imaging box device (W 7.5 cm × H 11.5 cm × L 6.5 cm) to avoid ambient light interference, which was attached camera of the smartphone. The distance between smartphone and the paper strip is 10 cm.

2.3 Fabrication of the paper strip biosensor

The fabrication process of the paper strip biosensor is shown in figure 1a by screening printing method using polylactic acid (PLA) to create hydrophobic regions. The chromatography paper was used as the base material for paper-based device. The paper strip consists of three parts. Firstly, the detection zone is designed to developing reaction for glucose analysis as the hydrophilic area. Next part is hydrophobic area, which is treated with PLA to separate hydrophobic and hydrophilic regions. Final part of the paper strip was fabricated for handle. This designed pattern was used to create the patterned screen. Subsequently, the fabricated patterned screen was placed onto a chromatography paper sheet. The 10 mL of 12 % (w/v) PLA solution is screen-printed onto the surface of chromatography paper, and the printed paper is dried at room temperature. The paper strip was coated with the polymer as the hydrophobic region and the area without polymer as the hydrophilic barrier. Finally, the paper was cut to give single pieces of the paper strip (0.5×3 cm) for use.

2.4 Procedure of colorimetric biosensor of glucose detection on paper strip

Figure 1b shows how the paper strip biosensor is used for analysis of glucose. The optimized procedure was determined as follows. Briefly, the first step is the addition of 1 µL of each 0.015 mg/mL GOD and 0.12 mg/mL HRP to the detection zone. After that, the 4 µL mixture solution between 8 mM phenol and 3 mM 4-aminoantipyrine were added. Subsequently, 4 µL of glucose solution or extracted rice sample was pipited to the detection zone of the paper strip biosensor. This strip was allowed to completely dry for 7 min at room temperature. The pink color was obtained on the detection zone. Then it was placed in the imaging box to take the reaction image by the smartphone. Finally, the glucose concentration was derived using self-written RGB color analysis software installed on the smartphone in the form of the Color Picker Application. The color intensity was obtained from the green channel of histogram. Because the channel provided the highest color intensity. The mean intensity value was calculated as the difference between the green value of the sample and the blank of detection. Subsequently, these values were used to calculate glucose concentration by using the calibration standard curve between the glucose concentrations vs color intensity.

2.5 Rice extraction

Ten samples of rice, each weighing one gram, are mixed with 1 mL of distilled water and homogenized in a beaker. This solution was incubated at room temperature for 50 min. After that, above mixture was filtrated using the filter paper No.1 and the supernatant was collected to further glucose analysis with the proposed assay.

3. Results and Discussion

3.1 Response of the colorimetric paper strip biosensor toward glucose

On the detection zone, the GOD catalyzes the oxidation of glucose producing gluconic acid and hydrogen peroxide. The hydrogen peroxide further reacts with phenol and 4-aminoantypyrine using HRP as catalyst. The pink color of producted 4-N-(p-benzoquinoneimine)–antypyrine in the detection zone can be easily detected by the naked eye (figure 2). As the results, intensity increased with the increasing of glucose concentration.

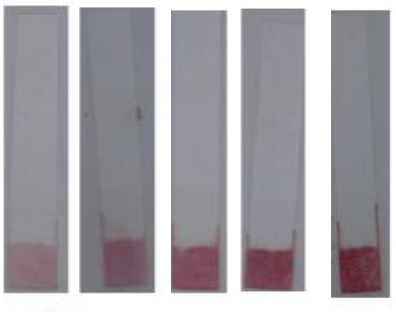

Figure 2 Images of colored products obtained from the paper strip biosensor response to different concentrations of glucose.

3.2 Optimization of parameters for the construction of the paper strip biosensor

The colorimetric paper strip biosensor in this work was based on the enzymatic reaction with chromogenic reagents. Thus, the important detection conditions including the concentrations of GOD, HRP, phenol and 4-aminoantypyrine. Moreover, pH and reaction time were optimized to improve colorimetric signal detection with the smartphone.

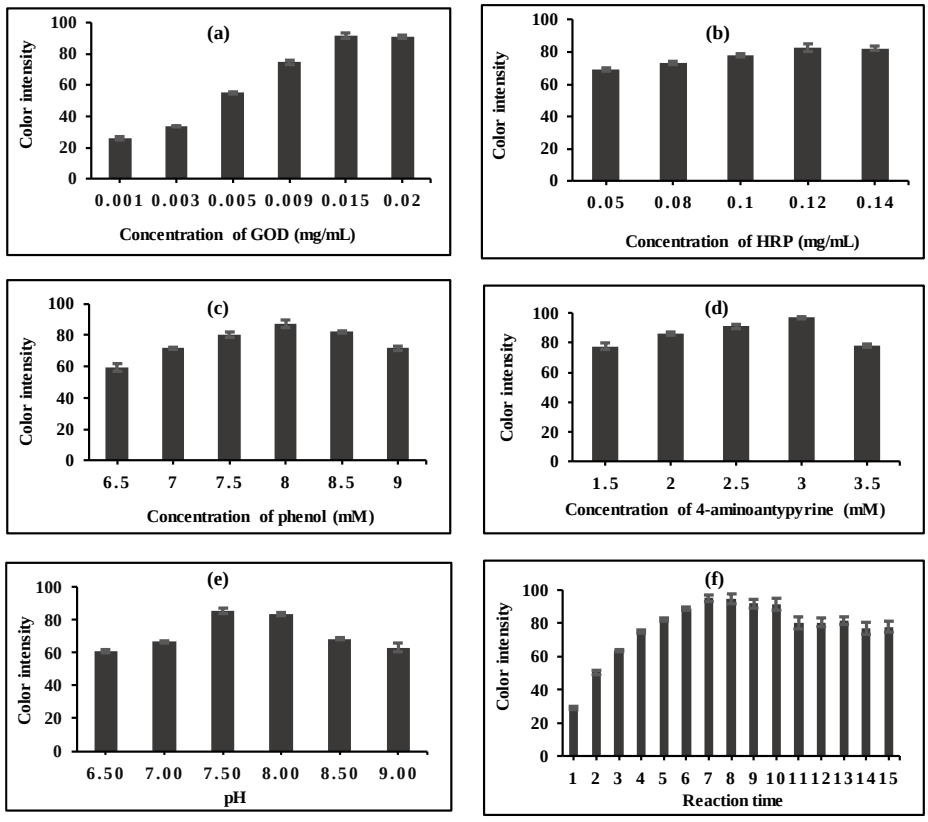

Figure 3 Optimization results; (a) concentration of GOD, (b) concentration of HRP, (c) concentration phenol, (d) concentration of 4-aminoantypyrine, (e) pH, and (f) reaction time.

Firstly, different concentrations of enzymes loaded in the paper strip biosensor (GOD from 0.001 to 0.02mg/mL) were studied keeping all other factors constant. This find out the conditions providing the best compromise between low enzyme loading and high color intensity, thus giving a cost-effective yet sensitive portable paper strip biosensor for glucose detection. When the concentrations were increased from 0.001 to 0.015 mg/mL, after 0.015 mg/mL, the color intensity displayed saturation without difference (figure 3a). Thus, 0.015 mg/mL was selected as optimal GOD concentration.

Figure 3b presents the result of a study on the effect of the HRP concentration. The color intensity increased from 0.05 to 0.12 mg/mL as the HRP concentrations increased, after which it curved toward a steady response at higher concentrations. In consideration of these results, the optimum HRP concentration of 0.12 mg/mL was selected and used to further studies.

Subsequently, an evaluation focused on the optimization of amounts of chromogenic reagents, including phenol and 4-aminoantypyrine. The investigation focused on evaluating the influence of phenol concentration on the color response of the paper strip biosensor within the range of 6.5 to 9 mM. The results of

the study are presented in figure 3c. The increase in phenol concentration initially resulted in an increase in color intensity, which was then followed by a decrease at concentrations over 8 mM. So, the paper strip biosensor for glucose detection were fabricated using phenol concentration as 8 mM.

To investigate changes in the paper strip biosensor responses due to different 4-aminoantypyrine concentrations, concentrations of 4-aminoantypyrine were studied between 1.5 and 3.5 mM. The results are given in figure 3d. The highest color intensity was obtained using 3 mM of 4-aminoantypyrine, so was used as the optimum concentration for the paper strip biosensor preparation.

To study the effect of the working pH of enzyme solution on the paper strip biosensor responses, experiments were carried out using phosphate buffer with different pH values (pH 6.50, 7.00, 7.50, 8.00, 8.50 and 9.00). The color intensity of the paper strip biosensor increased with increase of the pH value, and the highest intensity was observed at pH 7.50 but diminished for higher pH values. As the result, the pH 7.50 was determined as the optimal value (figure 3e).

In addition, the effects of reaction time on glucose detection were investigated, and the results are shown in figure 3f. As a result of the reaction time effect, the reaction time effect become significantly from 1 to 7 min, and then incline to a steady value for longer time. This indicated that the interaction of glucose and substrate reagents had reached equilibrium. Therefore, the reaction time of 7 min was selected as an optimal value for subsequent studies.

Based on the optimization experiments, optimum conditions of 0.015 mg/mL GOD, 0.12 mg/mL HRP, 8 mM phenol, 3 mM 4-aminoantypyrine, pH 7.50 and 7 min for reaction time were used for the following experiment.

3.3 Analytical performance of the paper strip biosensor

Under the optimal detection conditions, the developed paper strip biosensor was established to detect different concentrations of glucose and the smartphone was applied for quantification analysis. The results were shown in figure 4a and figure 4b. The results were found that, with increasing glucose concentrations, the color intensity gradually increased. The color intensity was proportional with the glucose concentration within the range of 0.5-10 mM (r = 0.9994). The limit of detection (LOD) (defined as 3 SD/m, where SD is the standard deviation for 10 blanks and m is the slope of the calibration curve) was 0.17 mM. In addition, the limit of quantitation (LOQ) of glucose detection was calculated to be 0.57 mM on the basis of 10 SD/m.

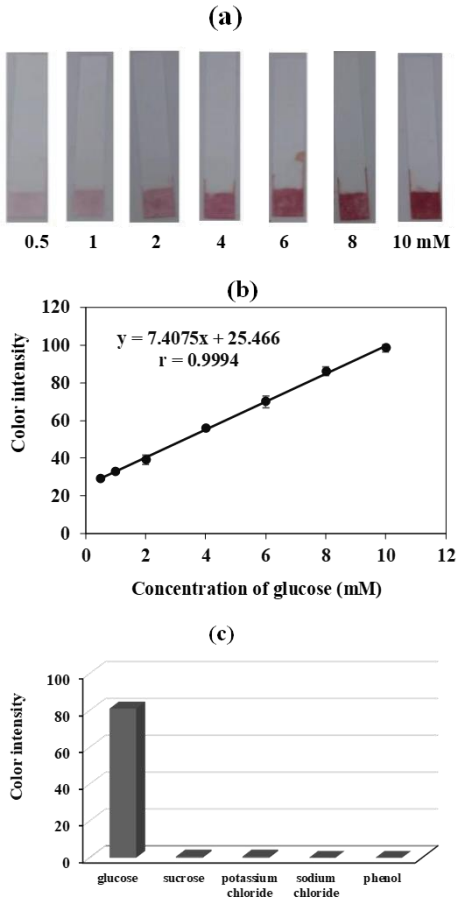

Figure 4 (a) Colourimetric responses of paper strip biosensor to different concentrations of glucose. (b) Calibration curve of glucose. (c) Selectivity of the paper strip biosensor with other interfering compounds.

The repeatability of the paper strip biosensor (n=7) was evaluated by detecting 10 mM glucose for 7 times, resulting a little change of color intensities with relative standard

deviation (RSD) of 0.94 %. Additionally, the reproducibility of the proposed sensor was assessed by using seven days with batches of the paper strip fabrications (n=7). The % RSD of the color intensities was calculated to be 2.84, demonstrating reasonable reproducibility.

In order to test the selectivity of the paper strip biosensor, sucrose, potassium chloride, sodium chloride and phenol that commonly existed in real samples with the same concentration of 10 mM were tested under the same conditions. As displayed in figure 4c, no significates color changes on detection zone were observed for all interfering compounds.

3.4 Analysis of glucose in rice

The paper strip biosensor was used to determine glucose in different rice cultivars including Khao Yellow Pathio, Khao Dawk Mali 105, Khao Gor Kor 14, Khao Gor Kor 57, Khao Gor Kor 77, Khao Gor Kor 79, Khao Thai Jasmine , Khao Gor Kor 31 , Hom Pathum and Bpraa Jeen, and the results were shown in Table 1. The results were found that the glucose contained in the range of 3.46-8.03 mg/kg. The hightest glucose levels obtained from Khao Gor Kor 41, whereas lowest are Bpraa Jeen.

Table 1 Detection of glucose in rice samples with the proposed paper strip biosensor (n=3).

Samples	Glucose (mg/kg)
Khao Yellow Pathio	7.91±0.09
Khao Dawk Mali 105	7.13±0.22
Khao Gor Kor 41	8.03±0.31
Khao Gor Kor 57	6.16±0.11
Khao Gor Kor 77	6.25±0.14
Khao Gor Kor 79	7.76±0.07
Khao Thai Jasmine	4.85±0.25
Khao Gor Kor 31	4.76±0.31
Hom Pathum	4.75±0.09
Bpraa Jeen	3.46±0.07

4. Conclusions

We fabricated a paper strip biosensor for the colorimeric detection of glucose in rice samples. GOD and HRP were used as biological sensing elements and a smartphone was uses as a detector. Compared with some conventional methods, the proposed paper strip biosensor exhibits the following remarkable advantages. (1) The paper strip biosensor can quickly detect glucose within 7 min. (2) The easy to use of the paper strip biosensor makes it does not require professionals and precision instruments. (3) The strategy can have used in on-site detection. Thereby, the developed paper strip biosensor can be applied for a new alternative for glucose analysis in rice samples.

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Declaration of Conflicting Interests

The authors declared that they have no conflicts of interest in the research, authorship, and this article's publication.

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