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A simplified and miniaturized glucometer-based assay for the detection of β-glucosidase activity*#

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Abstract: β-Glucosidase activity assays constitute an important indicator for the early diagnosis of neonatal necrotizing enterocolitis and qualitative changes in medicinal plants. The drawbacks of the existing methods are high consumption of both time and reagents, complexity in operation, and requirement of expensive instruments and highly trained personnel. The present study provides a simplified, highly selective, and miniaturized glucometer-based strategy for the detection of β-glucosidase activity. Single-factor experiments showed that optimum β-glucosidase activity was exhibited at 50 °C and pH 5.0 in a citric acid-sodium citrate buffer when reacting with 0.03 g/mL salicin for 30 min. The procedure for detection was simplified without the need of a chromogenic reaction. Validation of the analytical method demonstrated that the accuracy, precision, repeatability, stability, and durability were good. The linear ranges of β-glucosidase in a buffer solution and rat serum were 0.0873–1.5498 U/mL and 0.4076–2.9019 U/mL, respectively. The proposed method was free from interference from β-dextranase, snailase, β-galactosidase, hemicellulase, and glucuronic acid released by baicalin. This demonstrated that the proposed assay had a higher selectivity than the conventional dinitrosalicylic acid (DNS) assay because of the specificity for salicin and unique recognition of glucose by a personal glucose meter. Miniaturization of the method resulted in a microassay for β-glucosidase activity. The easy-to-operate method was successfully used to detect a series of β-glucosidases extracted from bitter almonds and cultured by *Aspergillus niger*. In addition, the simplified and miniaturized glucometer-based assay has potential application in the point-of-care testing of β-glucosidase in many fields, including medical diagnostics, food safety, and environmental monitoring.

Key words: Glucometer-based assay; β-Glucosidase; Activity detection
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1 Introduction

β-Glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) is a type of hydrolytic enzyme that plays an important role in the biotransformation of components (Wan et al., 2014; Peng et al., 2015) and flavor enhancement of both fruit juice and wine (Sharp et al., 2017; de Ovalle et al., 2018). In recent years, the extremely diverse physiological functions of β-glucosidase have been reported, including glucoside ceramide catabolism in human tissues and cell walls (Patro et al., 2014), defense against pathogens in plants, and utilization of oligosaccharide substrates by bacteria (Gueguen et al., 1997). The activity of β-glucosidase is often used as an indicator to demonstrate qualitative changes in organisms. It has been reported as a potential biomarker for the early diagnosis of neonatal necrotizing enterocolitis (NEC) (Chen et al., 2012;

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Gómez-Chaparro Moreno et al., 2016) and as an alternative therapeutic strategy to overcome chemoresistance in breast cancer (Zhou et al., 2017). Furthermore, some Chinese herbal medicines, such as *Semen Armeniacae Amarum* (Zhao LY et al., 2017), *Chrysanthemum morifolium* (Zuo et al., 2015), and *Scrophularia* root (Yu et al., 2017), are studied by detecting β-glucosidase activity to reveal the mechanisms of active ingredient hydrolysis during periods of processing and storage. Therefore, a simple, low-cost, and rapid method for the detection of β-glucosidase activity is much desired.

Traditional analytical methods for detecting β-glucosidase activity include the dinitrosalicylic acid (DNS) assay (Hu et al., 2013) and *p*-nitrophenyl-β-D-glucopyranoside (pNPG) spectrophotometric assay (Rather et al., 2010; Strahsburger et al., 2017). The mechanisms of these two methods are based on the liberation of monosaccharides and subsequent formation of colored or fluorescent substances, as shown in Table 1. Although they are commonly adopted in many experiments, both methods are time- and reagent-consuming, complex in operation, and require expensive instruments and trained personnel, which are not feasible for point-of-care testing (POCT) and impede their broad application in resource-limited regions.

A personal glucose meter (PGM), one of the most widely used diagnostic devices, opens a new horizon for on-site and real-time molecule detection owing to its portability, low cost, ease of use, small amounts of samples required, and high selectivity. A PGM is extensively developed to measure a variety of targets, such as glycosidases (Gurale et al., 2016), metal ions (Su et al., 2013; Zhang et al., 2015), proteins (Zhang et al., 2016; Zhao YT et al., 2017), and pathogenic bacteria (Wang et al., 2015). However, a PGM to detect β-glucosidase activity has not yet been developed.

With the aim of simplifying and miniaturizing an analytical method, in the present study we developed a glucometer-based assay for detection of β-glucosidase activity. First, experimental conditions of β-glucosidase were optimized. Second, methodological validation was evaluated to prove the feasibility of the proposed assay. Finally, we successfully applied the novel method to detect a series of β-glucosidases extracted from bitter almond and processed bitter almond, or cultured by *Trichoderma reesei*, *Trichoderma viride*, and *Aspergillus niger*. More importantly, the present paper provides an alternative strategy that could serve as the basis for the advanced development of sensitive point-of-care β-glucosidase detection in many fields, including medical diagnostics, food safety, and environmental monitoring.

2 Materials and methods

2.1 Reagents and standards

D-Glucose anhydrous (Lot number: 110833-201506, quantitative reference material, purity 99.9%) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Shanghai, China). D-(−)-Salicin (Lot number: G1522057, purity 99%) was purchased from Aladdin Industrial Corporation (Shanghai, China). Polyvinyl pyrrolidone (PVP; Lot number: 20170308) was provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bitter almond (Lot number: 121101) was purchased from Anhui Fengyuan Tongling Medicinal Materials Co., Ltd. (Anhui, China) and was further developed into processed bitter almond. β-Glucosidase (powder) was obtained from Shanghai Baofeng Biochemical Co., Ltd. (Shanghai, China) and Sigma-Aldrich Co. (Lot number: BCBR8001V, 7.6 U/mg, USA). β-Dextranase (Lot number: JL0719LA13, biological reagent (BR), 50 U/mg), snailase (Lot number: J07D6t7229, BR, 90%), β-galactosidase (Lot number: ZJ0703NA13, BR, 250 U/mg), hemicellulase (Lot number: YY10533B, activity ≥20000 U/g), and pNPG (Lot number: F29J9 Y53832) were provided by Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai, China). All other reagents used in this study were of analytical grade. Distilled water was used for all experiments. Preparations of DNS reagent and glucose calibration standards are shown in Data S1 and Data S2, respectively.

Table 1 Color formation mechanisms of the DNS and pNPG assays

Method	Reaction substrate	Final color compound
DNS	3,5-Dinitrosalicylic acid	3-Amino-5-nitrosalicylic acid
pNPG	4-Nitrophenyl-β-D-glucopyranoside	*p*-Nitrophenol

2.2 Apparatus

Enzyme extraction was performed using a refrigerated centrifuge (ST16R, Thermo Fisher, USA) and vortex mixing apparatus (VORTEX 3, IKA, Germany). Enzyme incubation was carried out using a thermostat water bath (DK-S26, Jinghong, Shanghai, China). A PGM (ACCU-CHEK Active, Roche, Germany) and ultraviolet spectrophotometer (Cary 8454 UV-Vis, Agilent Technologies, USA) were employed to detect β-glucosidase activity.

2.3 Enzyme extraction

The extraction of crude almond β-glucosidase was carried out by mixing 0.5 g of bitter almonds and 0.5 g of PVP with citric acid-sodium citrate buffer solution (0.05 mol/L, pH 5.0), and grinding into a homogenate on an ice bath. The homogenate was subsequently centrifuged at 10000 r/min and then 12000 r/min for 10 min at 4 °C. The resulting supernatant solution was collected and diluted to a final volume of 10 mL using the same buffer solution and thereafter stored at 4 °C.

2.4 Enzyme culture

T. viride QM9414, *T. viride* 3.316, *A. niger* M85, and *A. niger* M92 were inoculated on separate potato dextrose agar (PDA) slants to grow fungal spores at 28 °C. Spores grown on the slants were washed and inoculated in minimal medium (MM) enzyme culture medium at 1×10^8 spores/L at 28 °C for 5 d. The fermentation broth was subsequently centrifuged at 10000 r/min for 10 min at 4 °C, with the supernatant collected as β-glucosidase fermentation broth.

2.5 Enzyme assay

The enzyme assay was performed by adding 0.5 mL of enzyme solution to 1.5 mL of 0.03 g/mL salicin solution preheated in a 50 °C water bath for 5 min. The reaction mixture was incubated at 50 °C for 30 min, followed by incubation in boiling water for 10 min to stop the reaction. The glucose liberated in the mixture was detected using a PGM as an indicator of β-glucosidase activity. One unit (U) of activity was defined as the quantity of β-glucosidase required to hydrolyze salicin and release 1 μmol of glucose per minute in the above conditions. Inactivated β-glucosidase was treated as the blank control. The experimental protocol design is shown in Fig. S1.

2.6 Optimization of experimental conditions of β-glucosidase

In order to evaluate the effect of single factors on β-glucosidase activity, different parameters were investigated, including type of buffer solution, pH value (from 3.5 to 8.0), substrate concentration (from 0.0025 to 0.03 g/mL), incubation temperature (from 40 to 80 °C), and incubation time (from 10 to 120 min) (Li et al., 2017).

2.7 Method validation

The linearity of the proposed method was assessed by analyzing calibration standards of different glucose concentrations (6.1608, 4.6206, 3.8505, 2.3103, 1.5402, and 0.7701 mg/mL). Accuracy was determined by a recovery study of glucose using the standard addition technique. Precision was expressed as a percentage of the relative standard deviation (%, RSD) of three different calibration standards repeated six times in one day, and three times in three days. Six crude almond β-glucosidase samples were replicated and analyzed. In addition, the method's stability, durability, and selectivity were all studied.

3 Results and discussion

3.1 Optimization of experimental conditions for β-glucosidase

Five reaction factors, including buffer solution, pH value, substrate concentration, incubation temperature, and incubation time, were independently investigated to confirm the optimal experimental conditions for β-glucosidase. The preliminary conditions were set as follows: incubation temperature of 50 °C, incubation time of 30 min, and substrate concentration of 5 g/L.

3.1.1 Effect of buffer solution and pH values on β-glucosidase activity

A range of pH values (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, and 8.0) of citric acid-sodium citrate buffer solution (0.05 mol/L) and citric acid-disodium hydrogen phosphate buffer solution (0.05 mol/L) were studied under the predetermined primary conditions. As shown in Fig. 1a, β-glucosidase activity was higher in the citric acid-sodium citrate buffer solution. A notable increase in activity was observed for pH values from

3.5 to 5.0, beyond which activity began to decrease. Therefore, citric acid-sodium citrate buffer solution (0.05 mol/L) at pH 5.0 was selected as the optimal extraction solvent for β-glucosidase.

3.1.2 Effect of incubation temperature on β-glucosidase activity

Temperature has an impact on enzyme activity. Inactivation of some enzymes always occurs at high temperatures. As shown in Fig. 1b, the maximum activity of β-glucosidase was 1.2963 U/mL when the temperature reached 50 °C. We thus chose 50 °C as the optimal incubation temperature for subsequent experiments.

3.1.3 Effect of incubation time on β-glucosidase activity

Time is another important factor influencing β-glucosidase activity. With time ascending from 10 to 120 min, β-glucosidase activity gradually increased (Fig. 1c). Taking efficiency into consideration, 30 min was adopted for subsequent experiments.

3.1.4 Effect of substrate concentration on β-glucosidase activity

Substrate concentrations ranging from 0.0025 to 0.03 g/mL were investigated. The maximum activity of β-glucosidase was 2.3182 U/mL when the substrate concentration was 0.03 g/mL. As shown in Fig. 1d, higher substrate concentrations resulted in the higher enzyme activity; however, substrate solutions with concentrations greater than 0.03 g/mL were suspensions that were inhomogeneous. Thus, a 0.03 g/mL salicin solution was used in subsequent assays.

To summarize, the optimal experimental conditions for β-glucosidase activity were as follows: extraction solvent of citric acid-sodium citrate buffer solution (0.05 mol/L, pH 5.0), substrate concentration of 0.03 g/mL, incubation temperature of 50 °C, and incubation time of 30 min.

3.2 Method validation

3.2.1 Linearity and calibration curve

Linearity is important for an analytical method in order to produce results that are directly proportional to the analyte concentration in a sample. For the current method, linearity was defined as the relationship between the concentration of the glucose calibration standards and the corresponding sugar content measured by a PGM. The assay was performed by combining 0.5 mL of each calibration standard and 1.5 mL of buffer solution in a boiling water bath for 10 min, followed by cooling to 25 °C for measurement. The calibration curve was linear in the range of 0.1925–1.5402 mg/mL, which was represented as: y=7.0851x−0.6003 (R^2=0.9992).

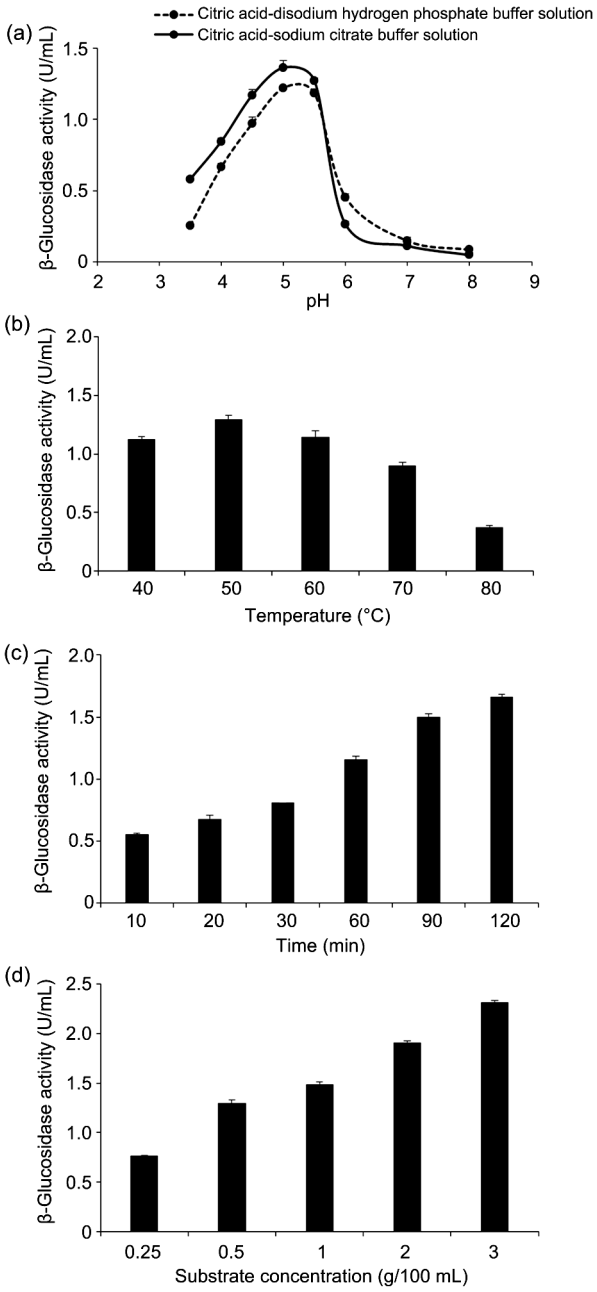

Fig. 1 Effects of different reaction factors on β-glucosidase activity

(a) Buffer solution types and pH values; (b) Incubation temperature; (c) Incubation time; (d) Substrate concentration. Data are expressed as mean±standard deviation (SD), n=3

3.2.2 Accuracy, precision, repeatability, stability, and durability of the proposed method

As shown in Tables 2 and 3, accuracy, precision, repeatability, stability, and durability of the proposed method performed well. Durability was evaluated by measuring the activity of crude almond β-glucosidase under optimal conditions with few changes. Prepared crude almond β-glucosidase samples were found to be stable when stored for 0, 4, 8, 16, and 24 h.

Table 2 Validation sheet and regression parameters of the proposed method

Parameter	Value
Accuracy[a] (*n*=3)	
80%	104.49%
100%	104.25%
120%	100.38%
Intraday precision (RSD)	
1.5402 mg/mL[b]	0.58%
0.9626 mg/mL	3.55%
0.1925 mg/mL	6.62%
Interday precision (RSD)	
1.5402 mg/mL[b]	0.49%
0.9626 mg/mL	1.79%
0.1925 mg/mL	5.97%
Repeatability[c]	
Mean	1.8339 U/mL
RSD	0.49%
Stability[d] (*n*=3)	
Mean	1.8429 U/mL
RSD	0.36%
LOD[e]	
Buffer solution	0.0873 U/mL
Rat serum	0.4076 U/mL

[a] Average recovery of three standard addition levels (80%, 100%, 120%) of β-glucosidase samples. [b] The concentrations of calibration standards were 1.5402, 0.9626, and 0.1925 mg/mL. [c] Average activity of six crude almond β-glucosidase samples and its relative standard deviation (RSD). [d] Average activity of one crude almond β-glucosidase sample at 24 h (0, 4, 8, 16, and 24 h) and its RSD. [e] LOD: limit of detection

Table 3 Durability of the proposed method (*n*=3)

Experimental condition[a]	Activity (U/mL)
Time (min)	
29	2.0307
31	2.0439
Temperature (°C)	
49	2.0307
51	2.0527
β-Glucosidase solution volume (mL)	
0.499	2.0307
0.501	2.0439

[a] Except for the alteration of one reaction parameter, all parameters in each group were the same as those in the optimal experimental condition

3.2.3 Selectivity of the proposed method

β-Dextranase, snailase, β-galactosidase, and hemicellulase are four widely used hydrolytic enzymes. In order to verify the specificity of the proposed method, it was applied to detect the four enzymes mentioned above. As per the results shown in Table 4, each of the four enzymes did not hydrolyze salicin to release glucose. This suggests that the presence of β-dextranase, snailase, β-galactosidase, and hemicellulase would not interfere with the determination of β-glucosidase activity.

Furthermore, three additional substrates, namely baicalin, geniposide, and icariin (0.01 g/mL), were investigated to compare the selectivity of the proposed method with the traditional DNS method (National Development and Reform Commission, 2003). Among the substrates, baicalin was hydrolyzed to liberate glucuronic acid, which could not be recognized by a PGM. As shown in Table 5, the selectivity of the proposed method was higher than that of the DNS method, which was mainly attributed to the unique recognition of glucose by a PGM. The principle of the obtained results was illustrated by Fig. 2.

3.2.4 Simplification and miniaturization of the proposed method

The DNS assay is most popular for the detection of β-glucosidase activity. However, it requires the reaction of sugar and DNS. To simplify the experimental procedure and simultaneously reduce the consumption of both sample and reagent, different

Table 4 Specificity of the proposed method

Competing substance[a]	Activity
β-Dextranase	E-2[b]
Snailase	E-2
β-Galactosidase	E-2
Hemicellulase	E-2

[a] The concentration of each substance is 2 mg/mL. [b] E-2 produces no glucose or the concentration of glucose is below 0.1 mmol/L

Table 5 Comparison of substrate selectivity between the proposed method and DNS method (*n*=3)

Substrate	Activity (U/mL)	
	Proposed method	DNS method
Baicalin		0.0311
Geniposide	0.3228	0.3410
Icariin	0.4063	0.3349

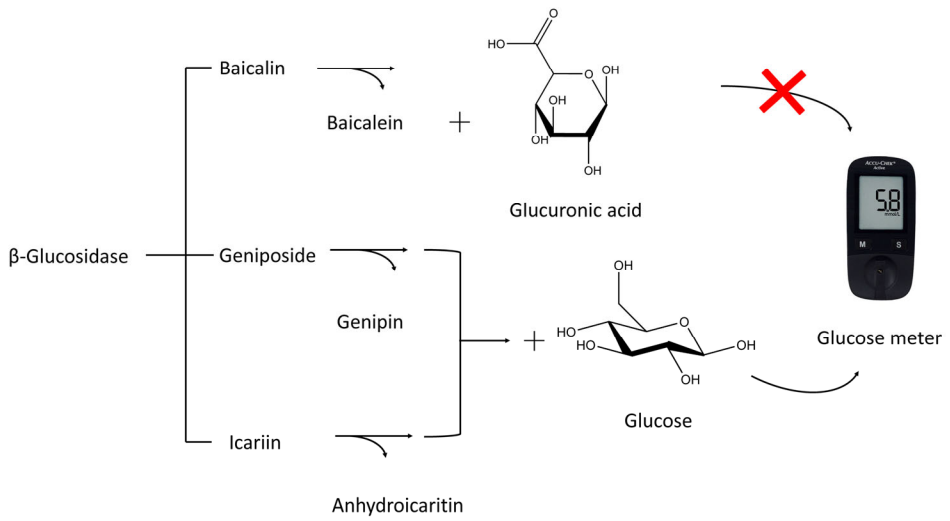

Fig. 2 Enzymatic hydrolysis of three different substrates

reaction mixture volumes (40 to 2000 μL) were studied. As shown in Table 6, the activity of crude almond β-glucosidase samples was similar at different volumes. The smallest volume of 40 μL was sufficient to detect β-glucosidase activity using the proposed assay. In addition, the proposed method was free from requiring a chromogenic reaction. Thus, the proposed method was shown to be a simpler, lower-cost, and more efficient method than the DNS assay.

3.3 Linear relationship between β-glucosidase activity and concentration in buffer solution and rat serum

A total of 5 mg of β-glucosidase powder (Sigma) was weighed and dissolved in 10 mL citric acid-sodium citrate buffer solution (0.05 mol/L, pH 5.0) and rat serum. These were used as the primary β-glucosidase solutions. The calibration solutions were diluted using the primary β-glucosidase solutions made in sodium citrate buffer solution and rat serum to 0.25, 0.125, 0.0625, 0.03125, and 0.015625 mg/mL and 0.125, 0.0625, 0.0417, 0.03125, and 0.015625 mg/mL, respectively.

As shown in Figs. 3a and 3b, as well as Fig. 4, the calibration curves in buffer solution and rat serum were linear when the activity ranges were 0.0873–1.5498 U/mL and 0.4076–2.9019 U/mL, respectively. The equations were y=13.484x−0.126 (R^2=0.9988) and y=22.874x−0.0862 (R^2=0.9953). These results indicate that the activity of β-glucosidase could be measured in both buffer solution and rat serum.

Table 6 Crude almond β-glucosidase activity with different volumes (n=3)

Volume (μL)		Activity (U/mL)
Enzyme solution	Substrate solution	
500	1500	1.9652
100	300	1.8645
50	150	1.8754
20	60	1.8899
10	30	1.8935

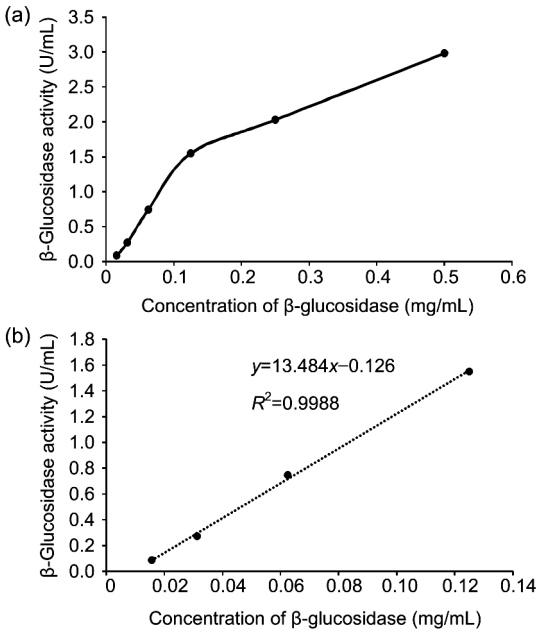

Fig. 3 Linear relationship between activity and β-glucosidase concentration in buffer solution
(a) β-Glucosidase activity at different concentrations; (b) Linear diagram of β-glucosidase activity

3.4 Application of the proposed method for the detection of different β-glucosidases

The analytical reliability and applicable potential of the proposed method were evaluated by testing a series of β-glucosidases. The activities of β-glucosidase (Baofeng), crude almond β-glucosidase, crude processed almond β-glucosidase, and β-glucosidase cultured by *A. niger* M92 were evaluated and found to be 0.3040, 1.8357, 0.3910, and 0.2432 U/mL, respectively (Table 7). By contrast, the concentrations of β-glucosidases cultured by *T. reesei*, *T. viride*, and *A. niger* M85 were too low to be detected. Furthermore, the statistical comparisons of the proposed method with the DNS and pNPG assays (Zhao LY et al., 2017) were evaluated and summarized in Tables 8 and 9. As shown in Table 8, the calculated *t* and *F* values were less than the tabulated values, indicating that there were no significant differences between the proposed method and the DNS assay. In Table 9, the calculated *t* value was more than the tabulated value, indicating that there was a significant difference between the proposed method and the pNPG assay when detecting the crude processed almond β-glucosidase.

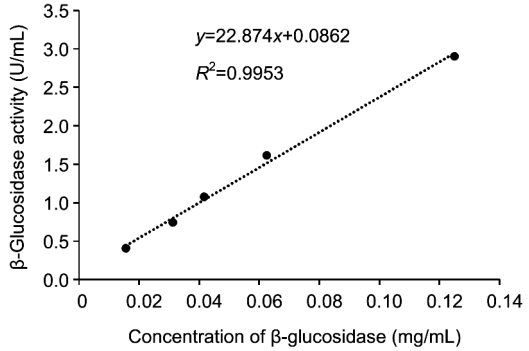

Fig. 4 Linear relationship between activity and β-glucosidase concentration in rat serum

Table 7 Activity of three different β-glucosidases (*n*=3)

β-Glucosidase	Activity (U/mL)	RSD (%)
Baofeng (2 mg/mL)	0.3040	2.05
Bitter almond	1.8357	0.68
Processed bitter almond	0.3910	4.10
A. niger M92	0.2432	2.44

4 Conclusions

The present study provided a simple, highly selective, and cost-effective glucometer-based strategy for the detection of β-glucosidase. Optimal experimental conditions for detecting β-glucosidase were determined and the proposed method was proved to have good accuracy, precision, stability, repeatability, durability, and selectivity. Owing to the direct determination of glucose by a PGM, limited quantities of reagents would be consumed. The experimental procedure was simplified because of the absence of a chromogenic reaction. The miniaturization of the analytical method ensured the simultaneous treatment for a large number of samples using a 96-well microplate, which further reduced the inter-batch error. Moreover, compared with the published paper (Gurale et al., 2016), this is the first application of a glucose meter for the detection of β-glucosidase activity. The study confirmed the initial hypothesis by

Table 8 Statistical comparison of the proposed method with the DNS assay (*n*=3)

Method	Crude almond β-glucosidase				β-Glucosidase solution (Baofeng, 2 mg/mL)			
	Mean (U/mL)	RSD (%)	*t*-test[a] (2.78)	*F* value[a] (19.00)	Mean (U/mL)	RSD (%)	*t*-test[a] (2.78)	*F* value[a] (19.00)
Proposed method	2.0307	0.75	0.62	0.03	0.3264	3.87	1.88	8.97
DNS method	2.0003	4.16			0.3517	2.10		

[a] The values in parenthesis are the corresponding theoretical values of *t* and *F* at *P*=0.05

Table 9 Statistical comparison of the proposed method with the pNPG assay (*n*=3)

Method	Crude processed almond β-glucosidase				β-Glucosidase solution (Baofeng, 0.5 mg/mL)			
	Mean (U/mL)	RSD (%)	*t*-test[a] (2.78)	*F* value[a] (19.00)	Mean (U/mL)	RSD (%)	*t*-test[a] (2.78)	*F* value[a] (19.00)
Proposed method	0.3910	4.10	4.86	11.7	0.1984	2.87	0.54	1.29
pNPG method	0.3442	1.36			0.2011	3.22		

[a] The values in parenthesis are the corresponding theoretical values of *t* and *F* at *P*=0.05

optimizing the reaction conditions, investigating the methodology, comparing the developed method with existing methods, and applying the developed method to different samples.

In summary, the significance of the present work is that the proposed method provides an alternative and improved approach for the POCT of β-glucosidase, and this method has applications in many fields, such as medical diagnostics, food safety, and environmental monitoring.

Contributors

Min-yi JIN performed the experiments and wrote the manuscript. Yi-shun YANG, Yue DING, and Jun-song LI edited the manuscript. Tong ZHANG and Gao-ren ZHONG designed the study.

Compliance with ethics guidelines

Min-yi JIN, Tong ZHANG, Yi-shun YANG, Yue DING, Jun-song LI, and Gao-ren ZHONG declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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List of electronic supplementary materials

Data S1 Preparation of the DNS reagent
Data S2 Preparation of the glucose calibration standards
Fig. S1 Experimental protocol design

中文概要

题　目：一种基于血糖仪的简便且微量的 β-葡萄糖苷酶活性检测方法

目　的：β-葡萄糖苷酶活性是新生儿坏死性小肠结肠炎早期诊断和药用植物有效成分改变的重要指标。现有活性检测方法操作时间长，试剂消耗大，且需要昂贵的仪器设备和专业的技术人员，不利于 β-葡萄糖苷酶的快速、实时检测。本文尝试构建一种基于血糖仪的酶活性检测方法，为实际样品中 β-葡萄糖苷酶活性的快速检测提供科学依据。

创新点：首次将便携式血糖仪引入 β-葡萄糖苷酶活性检测中，通过酶反应条件优化、方法学考察、实际样品中的应用以及现有方法的比较，建立 β-葡萄糖苷酶活性检测新方法。

方　法：采用单因素试验，以酶反应溶液、pH 值、反应温度、反应时间和底物浓度为指标，优化酶活性测定条件；通过考察精密度、稳定性、重复性、加样回收率、专一性等指标验证方法的可行性；通过实际样品中新方法的应用以及与现有二硝基水杨酸（DNS）法和 4-硝基苯基-β-D-吡喃葡萄糖苷（pNPG）法的比较，研究新方法在实际样品检测中的适用性。

结　论：酶活性测定最优条件为以柠檬酸−柠檬酸钠缓冲液（0.05 mol/L，pH 5.0）为反应溶液，0.03 g/mL 水杨苷为底物，在 50 °C 条件下，反应 30 min。新方法具有良好的精密度、准确性、重复性、稳定性和耐用性，且不受 β-葡聚糖酶、蜗牛酶、β-半乳糖苷酶、半纤维素酶和葡萄糖醛酸的影响，专一性较强。β-葡萄糖苷酶在缓冲液和大鼠血清中线性关系良好，线性范围分别为 0.0873~1.5498 U/mL 和 0.4076~2.9019 U/mL。该方法已成功应用于苦杏仁、燀苦杏仁、酶制剂以及黑曲霉培养的一系列 β-葡萄糖苷酶活性检测中，结果与现有方法所测的基本相符。

关键词：血糖仪；β-葡萄糖苷酶；活性检测