

Biofunctional characteristics of banana peel dietary fibre (BPDF) and its associated *in vitro* antidiabetic properties

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Abstract

The potential applications of banana peel waste can resolve environmental issues; however, the potentials of banana peels as antidiabetic remain unexplored. Therefore, the present work was carried out to investigate the biofunctional and surface properties of banana peel dietary fibre (BPDF) and its enzyme inhibitory activities. The water holding capacity (WHC), oil holding capacity (OHC), swelling capacity (SC), and glucose absorption capacity (GAC) were measured, and the glucose retardation index (GDRI) was analysed. The inhibitory effect of BPDF against α -amylase activity was also observed. The findings showed that the WHC (0.7 g/g), OHC (0.3 g/g), SC (0.73 mL/g), GDRI (6.58 - 31.72%), and GAC (0.162 - 19.211 mmol/g) of BPDF could have the potential in regulating diabetes, and explain the physiological effects of dietary fibre. The surface morphology of BPDF was analysed using scanning electron microscope. Interestingly, BPDF hampering effects on the diffusion of glucose through α -amylase inhibitory activity with IC_{50} 8.9 μ g/mL was found to be comparable to acarbose (IC_{50} 8.6 μ g/mL), thus showing potential in lowering postprandial blood glucose (type 2 diabetes mellitus).

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Keywords

banana peel, dietary fibre, biofunctional properties, glucose diffusion, α -amylase inhibitory activity

Introduction

Diabetes mellitus (DM) type 2 is generated by an imbalance insulin emission and glucose assimilation (Afrisham *et al.*, 2015). This consequently leads to short- and long-term health complications, and in certain cases could lead to death among younger patients (Olokoba *et al.*, 2012). Around 387 million diabetic patients have been accounted for in 2014 internationally, and two and a half million of them are Malaysian. In this regards, antidiabetic potentials from various plants have been studied, of which plant compounds such as carotenoids, flavonoids, polyphenols, terpenoids, alkaloids, and glycosides have been found to possess antidiabetic effects (Coman *et al.*, 2012; Etxeberria *et al.*, 2012; Lim and Loh, 2016; Adekola *et al.*, 2017).

Dream banana (*Musa acuminata* cv. P. Berangan) is among Malaysian mainstream industrial banana varieties. As compared to the pulp, the peels contain higher amounts of antioxidant compounds such as vitamins (A and C), carotenoids, and minerals (Pereira and Maraschin, 2015; Tsamo *et al.*, 2015), and

starch and dietary fibre components (Ramli *et al.*, 2010). According to Arunakumara *et al.* (2013), the fibre content in banana peel consists mostly of pectin (10 - 12%), followed by lignin, cellulose, and hemicellulose (6 - 9.6%). In addition, Chauhan *et al.* (2010) reported that pectin from banana peel extract displayed hypoglycaemic activity in ordinary and streptozotocin-induced diabetic mice by activating the secretion of insulin and reducing the glycogen content in the mice.

With regards to plant dietary fibre, their properties such as water-holding capacity (WHC) and swelling capacity (SC) have been useful to understand their physiology which is related to the permeable matrix structure formed by polysaccharide chains that can hold a lot of water through hydrogen bonds (Kethireddipalli *et al.*, 2002). The WHC is the limit of a wet material to hold water when exposed to an external centrifugal gravity power or pressure (Sharoba *et al.*, 2013), while the SC refers to the proportion between the volume of fibre and the heaviness of fibres (Ma and Mu, 2016). Other than that, the oil holding capacity (OHC) is related to the compound structure of the plant

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polysaccharides, and relies on the surface properties, overall charge density, thickness, and hydrophobic nature of the fibre particles (Garau *et al.*, 2007; Fernández-López *et al.*, 2009). The glucose absorption capacity (GAC) is the ability of the plant fibres to adequately bind glucose even at lower concentration and retard its transport across the intestinal lumen (Bhingé *et al.*, 2017). This subsequently inhibits the postprandial hyperglycaemia. Glucose dialysis retardation index (GDRI) is dependent on the retardation of glucose diffusion. It is a basic *in vitro* index to predict the effect of a fibre on the delay in glucose absorption in the gastrointestinal tract (Bhingé *et al.*, 2017).

The regulation of sugar hydrolysis into glucose substantially helps in the management of hyperglycaemia (Jumepaeng *et al.*, 2013; Lim and Loh, 2016). The use of plants is well-established in bringing down the blood glucose level and controlling hyperglycaemia by repressing the activity of α -amylase and α -glucosidase (El-Beshbishy and Bahashwan, 2012; Lim and Loh, 2016). Acarbose is an example of α -amylase and α -glucosidase inhibitor usually used in slowing down the digestion of carbohydrates (Sales *et al.*, 2012). However, the use of acarbose has been associated with side effects such as abdominal discomfort, flatulence, and diarrhoea. Therefore, there is a need for safer antidiabetic drugs to control hyperglycaemia. To the best of our knowledge, the investigation of banana peel as antidiabetic is still insufficient. Hence, in the present work, we investigated the functional properties of banana peel dietary fibre (BPDF) and its potential antidiabetic properties.

Materials and methods

Sample preparation

The banana fruits used in the present work were obtained from Giant supermarket at The Mines, Selangor, Malaysia. The preparation of the banana peels was carried out according to the method described by Fatemeh *et al.* (2012). The banana peel extracts were centrifuged at 3000 g for 15 min, followed by concentrating the supernatant using a rotary evaporator at 50°C. The BPDFs were collected and subjected to freeze-drying. The extracted BPDFs were stored at -20°C to maintain its bioactivity and avoid contamination until further analysis.

Biofunctional properties

Water holding capacity (WHC) and oil holding capacity (OHC)

The WHC and OHC of BPDF were measured

following the methods described by Sangeethapriya and Siddhuraju (2014) with slight modification. Firstly, 0.5 g of sample was added to 10 mL of distilled water or refined sunflower oil, and the mixture was left for 60 min at room temperature before centrifuging at 3000 g for 15 min. The supernatant was discarded, and the residue was weighed (x). The WHC and OHC were expressed as the amount of water or oil retained per milligram of mucilaginous sample (mg/mg) using Eq. 1. Each experiment was conducted in triplicates.

$$\text{WHC or OHC (mg/mg)} = \frac{x - 0.5}{0.5} \quad (\text{Eq. 1})$$

Swelling capacity (SC)

The SC of BPDF was measured following the method described by Sangeethapriya and Siddhuraju (2014). Firstly, 0.5 g of mucilaginous sample was added to 10 mL of distilled water, and the volume of solid was recorded (x). The mixture was left at room temperature for 18 h, and the bed volume was recorded (y). The experiment was prepared in triplicates. The SC was expressed as millilitre per gram of mucilaginous sample (mL/g), and calculated using Eq. 2:

$$\text{SC (mL/g)} = \frac{y - x}{0.5} \quad (\text{Eq. 2})$$

Glucose absorption capacity (GAC)

The GAC of BPDF was measured following the method described by Sangeethapriya and Siddhuraju (2014) with slight modification. A glucose solution (100 mL) with concentrations from 10 to 200 mmol/L was mixed with 1 g of sample before incubating at 37°C for 6 h. After reaching an equilibrium, the sample was centrifuged at 4000 g for 20 min. The glucose content in the supernatant was determined by using a glucose assay kit (Sigma-Aldrich, USA), and the measurement was prepared in triplicates. Guar gum and xanthan gum were evaluated for comparison. The absorbed glucose was calculated as the amount of glucose retained by the sample (mmol/g of sample) using Eq. 3:

$$\text{Glucose bound (mmol/g of sample)} = \frac{G1 - G6}{\text{Weight of sample} \times \text{Volume of solution}} \quad (\text{Eq. 3})$$

where, G1 = initial concentration, and G6 = concentration after 6 h incubation.

Glucose dialysis retardation index (GDRI)

The GDRI of BPDF was measured as a function of time following the method described by Sangeethapriya and Siddhuraju (2014) with slight

modification. Firstly, 0.5 g of mucilaginous sample was mixed with 25 mL glucose solution (50 mmol/L), and the mixture was dialysed against 100 mL distilled water at 37°C, using the dialysis membrane with cut-off molecular weight of 12,000 Da. The glucose content in the dialysate was measured after 30, 60, 90, and 120 min by using a glucose assay kit, and the measurement was prepared in triplicates. Guar gum and xanthan gum were evaluated for comparison. A control test was carried out without the addition of fibre. The GDRI was calculated using Eq. 4:

$$\text{GDRI} = 100 - \left[\frac{\text{Glucose content in the sample with fiber}}{\text{Glucose content of the control}} \times 100 \right] \quad (\text{Eq. 4})$$

Surface morphology

The surface morphology analysis was performed using a Scanning Electron Microscopy with Energy Dispersive X-ray (SEM–EDX) (Jeol Ltd., Japan) at the Institute of Bioscience (IBS), UPM.

α -Amylase inhibition assay

The inhibitory effect of BPDF against α -amylase action was measured according to the 3,5-dinitrosalicylic acid (DNSA) technique described by Marikkar *et al.* (2016) with slight modification. Firstly, the sample was diffused in methanol, and mixed with sodium buffer (pH 6.9) at a series of concentration (12.5 to 600 $\mu\text{g/mL}$). The BPDF (200 μL) was then mixed with 200 μL of *Bacillus licheniformis* α -amylase, and left at 30°C for 10 min. Each tube was added with 200 μL of the starch solution (1% in a buffer (w/v)), and the reaction was inhibited by adding 200 μL DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution), and further heated for 10 min in a water bath at 80°C. The mixture was then diluted with 5 mL of distilled water before reading absorbances at 540 nm using a UV-visible spectrophotometer. The experiment was prepared in triplicates. The α -amylase activity (inhibition %) of BPDF, quercetin, and acarbose were estimated using Eq. 5:

$$\text{Inhibition (\%)} = \left\{ \text{Ac} - \frac{(\text{As} - \text{Ab})}{\text{Ac}} \right\} \times 100 \quad (\text{Eq. 5})$$

where, Ac = absorbance of control, Ab = absorbance of blank, and As = absorbance of sample.

Statistical analysis

The experimental data were analysed using the analysis of variance (ANOVA), and presented as mean \pm standard deviation (SD) of triplicates ($n = 3$).

Means were considered significant when $p < 0.05$. The statistical analysis was performed using GraphPad Prism 7.00 for Windows.

Results and discussion

The biofunctional properties of BPDF (WHC, SC, OHC) are shown in Table 1. The banana peel showed hydration properties. The hydration properties of soluble dietary fibre hinder the ingestion of macronutrients which expands the insulin affectability. The WHC of BPDF was observed to be 0.7 g/g, which was lower than that in the dietary fibre of bambangan peel (11.6 g/g) (Abdulrahman *et al.*, 2011), pitaya peel (54.20 g/g) (Zhuang *et al.*, 2012), and *Z. mauritiana* mucilage (25.21 g/g) (Sangeethapriya and Siddhuraju, 2014).

Table 1. Functional properties of banana peel dietary fibre (BPDF).

Parameters	Value
Water holding capacity (WHC) (g/g)	0.7 \pm 0.14
Oil holding capacity (OHC) (g/g)	0.35 \pm 0.07
Swelling capacity (SC) (mL/g)	0.73 \pm 0.23

Values are mean \pm standard deviation of triplicate ($n = 3$).

The ability to increase in mass after retaining water is one of the important biofunctional properties of fibres. The SC of the BPDF was 0.73 mL/g, which was also lower than pitaya peel (18.7 mL/g) (Zhuang *et al.*, 2012), bambangan peel (50.63 mL/g) (Abdulrahman *et al.*, 2011) and *Z. mauritiana* mucilage (19.34 mL/g) (Sangeethapriya and Siddhuraju, 2014). The soluble dietary fibres in the cell wall of plants have hydrophobic polysaccharides that could influence the WHC and SC. The high WHC of dissolvable fibre affects their consistency; where the thick fibre in the intestinal substance decreases the ingestion of glucose in the gut which lessens the postprandial blood glucose level and is useful for diabetic patients.

Dietary fibre, which can hold oil, can bind bile acids and increase their discharge. This subsequently decreases the plasma cholesterol. In addition, the OHC of BPDF (0.35 g/g) was found to be much lower than that in bambangan peel (3.33 g/g) (Abdulrahman *et al.*, 2011), pitaya peel (2.65 g/g) (Zhuang *et al.*, 2012), and *Z. mauritiana* mucilage (12.53 g/g) (Sangeethapriya and Siddhuraju, 2014).

The impact of dietary fibre through an assimilation of glucose was observed in the present work. The value predicts the activities of fibre in reducing glucose in the gastrointestinal tract.

Figure 1 shows the glucose absorption capacity (GAC) of BPDF which ranged from 0.162 to 19.21 mmol/L at various concentrations (10 - 200 mmol/L). The glucose bound to these dietary fibres in a concentration-dependant manner. It was shown that the BPDF could retain the glucose in the intestinal lumen even at lower concentration (10 mmol/L). Initially, the GAC of BPDF was significantly lower than xanthan gum and guar gum at lower concentration of glucose (10 and 25 mmol/L). The glucose assimilation limit of BPDF was then observed to significantly increase at higher concentration of glucose (50, 100, and 200 mmol/L), and was comparable to xanthan gum and guar gum.

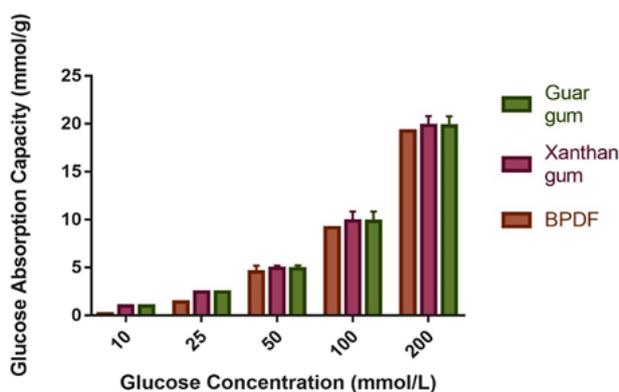


Figure 1. Glucose concentration (mmol/L) versus glucose absorption capacity (mmol/g). Error bars indicate standard deviation of triplicate ($n = 3$). BPDF = banana peel dietary fibre.

The effects of BPDF on the glucose movement across the dialysis membrane are shown in Figure 2. Overall, the BPDF showed an increase in the glucose amount found in the dialysate from 30 to 120 min, and the diffusion of glucose was time-dependent. As the time increased from 30 to 120 min, the glucose content in the dialysate of BPDF increased from 320.6 to 327.4 μmol . The control showed higher result (343.0 to 508.8 μmol) as compared to the sample which showed a reduced amount of diffused glucose. GDRI is a helpful *in vitro* index to determine the outcome of fibre on the

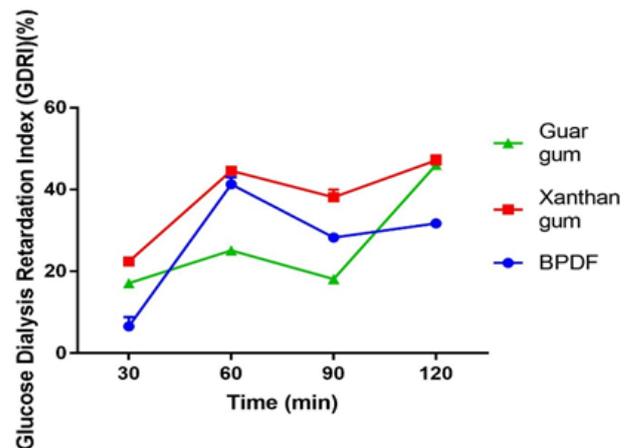


Figure 2. Time (min) versus glucose dialysis retardation index (%). Error bars indicate standard deviation of triplicate ($n = 3$). BPDF = banana peel dietary fibre.

hindrance of glucose assimilation in the gastrointestinal tract (Sangeethapriya and Siddhuraju, 2014). The GDRI of BPDF was the highest at 60 min (41.25%), comparable to the xanthan gum. The soluble dietary fibre produces viscous gel in aqueous solution that has the mechanism to retain glucose. This phenomenon decreases the diffusion rate of glucose, and may have a potential benefit of controlling the blood glucose level.

The morphological characteristics of banana peel at different magnifications are shown in Figure 3 (A-C). SEM images showed many oval granules with porous surface of BPDF. The available regiochemistry of the surface layer is expected to play a role in the adsorption or binding of molecules which accounts for some of the physiological effects of BPDF. The microstructure of BPDF with holes or pores was believed to promote easy diffusion of glucose and other sugars (Wang *et al.*, 2017), thus helping to reduce the absorption of those components, and control blood glucose level.

The *in vitro* α -amylase inhibitory activity of BPDF is shown in Figure 4. The breakdown of carbohydrates by the inhibition of α -amylase and

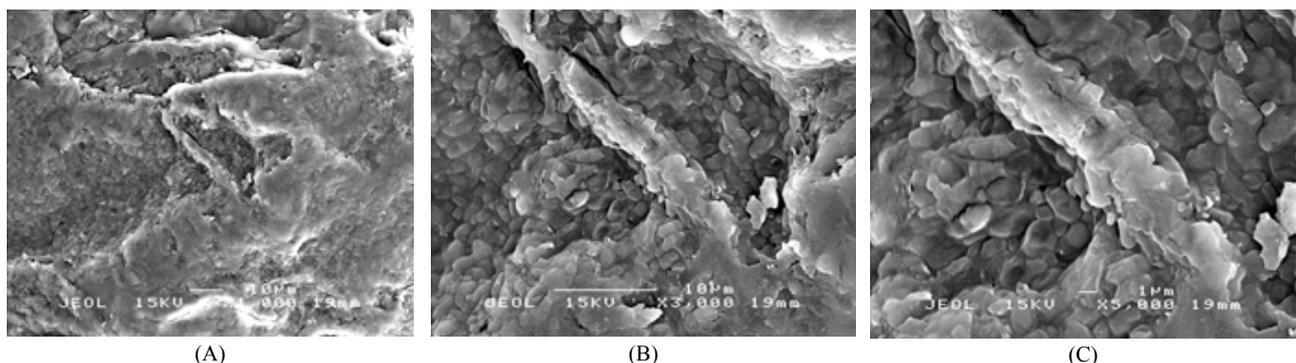


Figure 3. Scanning Electron Micrograph (SEM) of BPDF at 1000 \times (A), 3000 \times (B), and 5000 \times (C) magnification.

α -glucosidase has been misused as a restorative measure in lowering postprandial hyperglycaemia. Pancreatic α -amylase is associated with the breakdown of starch into disaccharides and oligosaccharides before being further hydrolysed by intestinal α -glucosidase into free glucose, afterward retained from the bloodstream. The inhibition of these enzymes would hinder the hydrolysis of sugar in the gastrointestinal tract, thus decreasing postprandial hyperglycaemia (Singh and Kumar, 2015). The IC_{50} values of acarbose and quercetin toward α -amylase action were observed to be 8.6 and 35.4 $\mu\text{g/mL}$, respectively. Interestingly, the IC_{50} value (8.9 $\mu\text{g/mL}$) of the BPDF was found to be comparable to acarbose, thus demonstrating similarity to synthetic drugs in performing the inhibitory potential against α -amylase. The natural protein inhibitors are probably going to offer an appealing remedial way to deal with the treatment of postprandial hyperglycaemia due to lower abdominal side effects arising from excessive inhibition of pancreatic α -amylase by synthetic drugs. In addition, according to Chauhan *et al.* (2010), the pectin-type polysaccharides from banana peel extract display the hypoglycaemic activity in ordinary and streptozotocin-induced diabetic mice by activating the secretion of insulin and reducing the glycogen content in the mice.

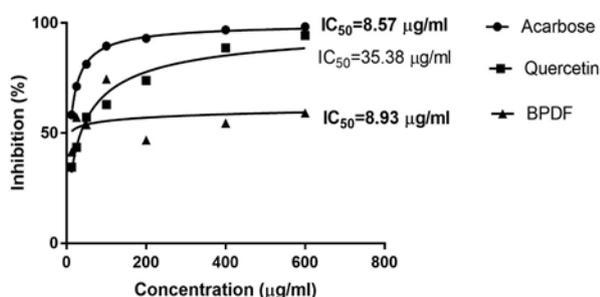


Figure 4. Dose-response curve of concentration ($\mu\text{g/mL}$) versus % inhibition α -amylase of BPDF.

Conclusion

The present work demonstrated the properties of BPDF including hydration properties, oil retention ability, and swelling capacity. As a matter of interest, the BPDF exhibited a significant increase in glucose absorption profile and glucose retardation index in delaying the glucose assimilation in the gastrointestinal tract, which is comparable to that of the standard guar gum and xanthan gum. In addition, the characteristics of BPDF microstructure illustrated the possible way for the diffusion of glucose and other sugars in controlling the potential indices of diabetes. With regard to its inhibitory potential, BPDF has

revealed the lowest IC_{50} value with promising α -amylase inhibition activity comparable to synthetic antidiabetic drugs, thus demonstrating its potential in lowering blood glucose level by impeding digestion of dietary carbohydrates. Further investigation is nevertheless warranted for fibre identification and their possible mechanism in managing and preventing diabetes mellitus.

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