

Modulation of fasting blood glucose by raw banana powder in alloxan-induced diabetic rats

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Abstract: This study aimed to observe the influence of raw banana powder (RBP) on fasting blood glucose (FBG), blood lipid and other biochemical indicators in type-2 diabetic rats and therefore to provide experimental evidences for developing suitable food from banana powder for diabetic patients. Eight Sprague-Dawley rats were selected randomly as the normal control group (NCG) before the experiment. After establishing type-2 diabetic rat models (11.1-16.7 mmol/L) by alloxan, 32 rats were divided into four groups: the diabetic control group (DCG, $n=8$), low-dose group (LDG, $n=8$), middle-dose group (MDG, $n=8$) and high-dose group (HDG, $n=8$). The LDG, MDG and HDG rats received gastric perfusion of RBP at the doses of 2 g/kg, 4 g/kg and 6 g/kg per day, respectively. After four weeks, oral glucose tolerance test was carried out in each group, and then the FBG level, blood lipid, insulin, short chain fatty acids content, pH value of colon content and other biochemical indicators of rats in each group were determined and compared among the groups. Results showed that the levels of FBG significantly decreased in the LDG (11.97 ± 0.83), MDG (8.95 ± 0.45) and HDG (9.28 ± 1.45), compared with their initial values (13.00 ± 1.25 , 13.68 ± 0.75 and 13.91 ± 0.80 , respectively). The FBG levels in these three groups were obviously lower than that in the DCG. However, there were no dramatic FBG changes in the NCG and DCG (5.77 ± 0.59 , 14.14 ± 0.72) compared with the initial stage (5.55 ± 0.23 , 13.93 ± 0.47). The RBP intervention increased insulin-sensitivity index and regulated postprandial blood glucose. Besides, RBP showed the positive effects on symptoms of type 2 diabetic rats, such as the reduction of weight gain and total cholesterol.

Keywords: raw banana powder, dietary fiber, resistant starch, fasting blood glucose, diabetic rats

DOI: 10.3965/j.ijabe.20130603.0012

Citation: Bai Y L, Yu M, Du B, Chen Q F, Yang G M. Modulation of fasting blood glucose by raw banana powder in alloxan-induced diabetic rats. *Int J Agric & Biol Eng*, 2013; 6(3): 94–102.

1 Introduction

Bananas are produced in large quantities in tropical and subtropical areas, but about one-fifth of all bananas harvested become culls. The first practical application

of culled bananas is to use the pulp for starch production or production of a low-cost banana flour ingredient. Banana powder has the potential to be a commodity starch because of its specific properties and its potential generation from low-cost cull bananas. Green banana pulp contains up to 70%-80% starch on a dry basis, a percentage comparable to that in the endosperm of corn grain and the pulp of white potato. In recent years, developing functional products from banana has become a focus^[1-4].

Worldwide, the population of diabetic people is predicted to rise from 171 million in 2000 to 366 million by 2030^[5]. When discussing strategies for metabolic control in diabetic patients, necessary life-style modifications should be considered^[6]. As for dietary habits, it has been reported that the intake of dietary fiber

Received date: 2013-05-24 **Accepted date:** 2013-06-17

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is linked to an increase in insulin sensitivity^[7-9]. The Institute of Medicine (IOM), the National Academy of Sciences, United States, has defined dietary fiber as the indigestible carbohydrates and lignin that are intrinsic and intact in plants, whereas functional fiber consists of the isolated non-digestible carbohydrates that have beneficial physiological effects on human beings^[10]. Accordingly, resistant starch (RS) is considered as a functional fiber by its indigestible nature and beneficial physiological effects. The RS escapes digestion in the human small intestine and is fermented in the colon with production of metabolically active short chain fatty acids (SCFA)^[11]. Moreover, RS-unlike nonresistant starch is not digested and therefore not absorbed as glucose in the small intestine of healthy humans^[12,13]. A reduction in both postprandial glycemia and insulinemia is expectable after the intake of RS, compared with digestible starch. The feature of RS may be good for the control of diabetes. Moreover, though mechanisms are similar to those exerted by dietary fiber, RS may influence the absorption amount and rate of other nutrients in the diet, glucose and fat, which may be useful for controlling glycemia or lipidemia.

Starches from different sources have diverse structures and consequently different resistance to enzymatic hydrolysis. The grade of resistance to digestibility among different starches generally correlates to their amylose content. Previous studies by our group have identified a high amylose-content and a high resistance to digestibility in this banana variety. The amylose content of Dongguan Dajiao starch was 22.9%. In vitro, the digestibility of Dajiao powder was no more than 15% within 4 hours.

Some previous studies have demonstrated that the improvement of RS or dietary fiber from different sources on the symptoms of diabetes, as a prebiotic, has hypoglycemic effects of inhibiting fat accumulation and reducing gall stones^[14-18]. Raw banana powder (RBP) is rich in dietary fiber, including not only soluble fiber, but also insoluble fiber. Although the beneficial influence of RS on the glycemic and insulin responses has been reported, there are few studies focusing on the effect of RBP on FBG of type-2 diabetic rats.

The banana powder used in this study was obtained from unripe banana (*Musa spp. ABB Group, Dongguan dajiao*) which is very cheap and highly yielded in Dongguan, Guangdong Province of China. The present study intended to investigate the effects of RBP on postprandial changes of glycemia, hormonal response and other biochemical indicators in type-2 diabetic rats.

2 Materials and methods

2.1 Test materials

The banana powder was provided by Foshan Jiaoye Biological Technology Co., Ltd., and was obtained from unripe (green) bananas (*Musa spp. ABB Group, Dongguan dajiao*). Briefly, after washing, the bananas were peeled, cut into 3-4 cm³ pieces and immediately macerated in the color protecting solution composed of sodium sulfite anhydrous (0.05%), citric acid (0.4%) and vitamin C (0.1%). The homogenate was then centrifuged at 4 000 r/min. Sediment was further purified by washing and centrifugation. The white starch sediment was dried in a spiral vibrofluidized bed model dryer at 40-45°C, passed through a 100 mesh screen after pulverization, and in the end stored at room temperature using the vacuum package. The yield of banana powder was 25%. The nutritional components in RBP were 8.6% of moisture content, 3.1% of protein, 0.51% of fat, 2.1% of ash, 62.3% of resistant starch and 12.3% of dietary fiber.

2.2 Animals and feeding

50 four-weeks-old male Sprague-Dawley rats were maintained on a normal diet for one week under the controlled environment. Then, eight rats were selected randomly as the normal control group (NCG). The other rats were fed with high glucose and high lipid diet (60% normal diet, 20% fat and 20% sucrose) for additional four weeks followed by intraperitoneal injection with 120 mg/kg alloxan in physiological saline. The NCG rats were fed with normal diet for four weeks, and then were injected with the same volume of physiological saline. FBG concentrations were analyzed using blood from rat tail vein. Thirty two diabetic rat models (11.1-16.7 mmol/L) were selected and unbiasedly divided into four groups: the diabetic control group (DCG, $n=8$), low-dose

group (LDG, $n=8$), middle-dose group (MDG, $n=8$) and high-dose group (HDG, $n=8$). The LDG, MDG and HDG rats received gastric perfusion of RBP at the doses of 2 g/kg, 4 g/kg and 6 g/kg RBP per day, respectively. In contrast, gastric perfusion of distilled water was given to the NCG and DCG rats. All rats were maintained on each diet for a 4-week period under the controlled environment (25-28°C, relative humidity 40%-70%, 12-hour light and dark cycle). Body weights were weighed every morning. Normal diet and high glucose/high fat diet were both prepared in Medical Experimental Animal Center of Guangdong Province, China. Normal diet was prepared on the basis of AIN-93G.

2.3 Blood sampling and colon content

Blood glucose was determined weekly by a glucometer using tail vein blood. After four weeks, animals were fasted overnight, and then blood was drawn from the abdominal aorta. The blood was allowed to clot and then centrifuged (8 min) to obtain serum. Blood serum samples were immediately frozen and conserved at negative 40°C until biochemical determinations. The analysis of cholesterol (CHO) and triglycerides (TG) was performed using the refrigerated serum on the next day. Fasting Insulin (FIns) concentrations were measured in plasma with rat insulin, INS ELISA Kits. To prevent enzymatic insulin degradation, special care was taken to avoid hemolysis during the blood sampling and manipulation. Four weeks later, OGTT was carried out in each group. In the test, rats were fasted for 5 hrs during the light period and gavaged with glucose solution (2.2 g glucose/kg of body weight). The blood glucose levels (BG_0 , BG_{15} , BG_{30} , BG_{60} , BG_{120}) were measured at 0, 15,

30, 60, and 120 min after the glucose gavage. The areas under the curve of glucose (AUCG) in each group were calculated. Insulin sensitivity index (ISI) was calculated and compared among groups by Homeostasis model assessment (HOMA). Colon contents were collected after intervention; the pH value of colon content was measured; and SCFA were detected with gas chromatography. The calculations are as follows:

$$AUCG = 1/2 \times (BG_0 + BG_{15}) \times 0.25 + 1/2 \times (BG_{15} + BG_{30}) \times 0.25 + 1/2 \times (BG_{30} + BG_{60}) \times 0.5 + 1/2 \times (BG_{60} + BG_{120}) \times 1$$

$$HOMA-ISI = 1/FBG \times FIns$$

$$HOMA-Insulin Resistance Index (IRI) = FBG \times FIns / 22.5$$

2.4 Analysis

RBP was analyzed for the presence of moisture (GB 5009.3-2010), ash (GB 5009.4-2010), protein (GB 5009.5-2010), fat (GB 5009.6-2003) and dietary fiber (GB/T 22224.3-2008) according to the National Standards of the People's Republic of China (GB). The determination of resistance in the banana starch flour was carried out based on the enzymatic method of AOAC 2002.02^[19]. Lipid (total cholesterol and triglycerides) levels were determined enzymatically using ELISA Kits purchased from Sigma Chemical Co. (St. Louis, MO).

The data were subjected to analysis of variance using the SPSS 1.7.3. The characteristics of the rats are presented as means \pm standard deviation. Statistical significance was defined as $P < 0.05$.

3 Results

3.1 Weight gains

Body weights of rats in each group were weighed every day in the intervention stage. The changes every week was showed in Table 1 after statistical analysis.

Table 1 Variations of rat body weight in diverse groups and time points after RBP intervention (g)

Group	HDG	MDG	LDG	DCG	NCG
Initial body weight	170.50 \pm 6.05	173.00 \pm 9.71	168.70 \pm 7.65	174.20 \pm 7.45	172.20 \pm 5.86
The 1 st week	188.50 \pm 8.67	185.00 \pm 9.71	184.50 \pm 6.75	186.20 \pm 6.75	182.20 \pm 7.85
The 2 nd week	223.75 \pm 9.58 ^b	216.25 \pm 7.99	222.75 \pm 10.80 ^b	223.25 \pm 8.32 ^b	210.25 \pm 6.09 ^a
The 3 rd week	254.00 \pm 10.10 ^b	236.00 \pm 11.87 ^a	252.25 \pm 3.78 ^b	258.00 \pm 8.31 ^b	230.00 \pm 5.83 ^a
The 4 th week	263.50 \pm 8.71 ^{ab}	258.25 \pm 11.89 ^{ab}	280.75 \pm 7.50 ^b	288.75 \pm 5.06 ^b	243.25 \pm 8.89 ^a
Added body weight	93.00 \pm 2.73 ^{ab}	85.25 \pm 2.12 ^{ab}	112.25 \pm 3.28 ^b	116.50 \pm 4.63 ^b	61.00 \pm 2.88 ^a

Note: HDG, high-dose group; MDG, middle-dose group; LDG, low-dose group; DCG, diabetic control group; NCG, normal control group; ^a, significantly different at the 0.05 probability compared with DCG in the same row; ^b, significantly different at the 0.05 probability compared with NCG in the same row.

The non-dose dependent effects of RBP on body weight were reported in type-2 diabetic rats. Consequently, the rats, who were fed with high glucose and high lipid diet and given 4-week gastric perfusion with RBP, gained less body weight than the DCG rats, while gained more weight than the NCG rats with the normal diet. Moreover, compared with the HDG and HLG, the effect of RBP on the MDG rats was more significant. That means that appropriate dosage of banana powder enhanced its effect on body-weight gain control. Various weight gains may contribute to different blood lipid responses observed.

3.2 Blood glucose control

FBG concentrations averaged (13.91±0.80) mmol/L (HDG), (13.68±0.75) mmol/L (MDG), (13.00±1.25)

mmol/L (LDG), (13.93±0.47) mmol/L (DCG) and (5.55±0.23) mmol/L (NCG) before gastric perfusion with RBP (Table 2). A significant effect of RBP on FBG in intervention groups was observed after the intervention. An obvious interaction between FBG and time was detected after the intervention in the MDG and HDG. After four-week intervention experiment, the levels of FBG in the LDG, MDG and HDG rats significantly decreased and were obviously lower than that in the DCG rats, but no obvious difference was found in the NCG (5.77±0.59) and DCG (14.14±0.72) rats, compared with the initial stage (5.55±0.23 and 13.93±0.47, respectively) (Table 2). It is worth mentioning that rats in the intervention groups did not change their high glucose and high lipid diet during the entire experimentation period.

Table 2 Variations of rat FBG levels in diverse groups and time points (mmol/L)

Group	HDG	MDG	LDG	DCG	NCG
Initial level	13.91±0.80 ^b	13.68±0.75 ^b	13.00±1.25 ^b	13.93±0.47 ^b	5.55±0.23 ^a
The 1 st week	12.03±1.26 ^b	11.30±1.55 ^{ab}	12.50±0.75 ^b	13.88±0.75 ^b	6.12±0.61 ^a
The 2 nd week	10.22±1.23 ^{ab}	10.18±1.48 ^{ab}	12.75±0.81 ^{ab}	14.10±0.82 ^b	5.87±0.60 ^a
The 3 rd week	9.11±1.24 ^{ab}	9.93±0.47 ^{ab}	12.25±0.41 ^b	13.50±0.51 ^b	5.50±0.80 ^a
The 4 th week	9.28±1.45 ^{abc}	8.95±0.45 ^{abc}	11.97±0.83 ^{abc}	14.14±0.72 ^b	5.77±0.59 ^a

Note: HDG, high-dose group; MDG, middle-dose group; LDG, low-dose group; DCG, diabetic control group; NCG, normal control group; ^a, significantly different at the 0.05 probability compared with DCG in the same row; ^b, significantly different at the 0.05 probability compared with NCG in the same row; ^c, significantly different at the 0.05 probability compared with initial level in the same column.

3.3 Insulin

Insulin resistance (IR) is the key mechanism of type-2 diabetes. IR is characterized as the tissue's inability to take up glucose in response to insulin. In the course of months or years, IR is accompanied by the increase in β -cell insulin secretion and by different complications known as the insulin resistance syndrome, which is associated to dyslipidemia, hypertension, hyperglycemia and cardiovascular disease^[20].

After four-week intervention experiment, there was no significant changes in the insulin level in the HDG and

MDG compared to the DCG, while a slight insulin decrease was observed, when compared to the NCG (Table 3). On the contrary, the insulin concentration of LDG significantly increased, compared to both the NCG and the DCG. As expected, ISIs measured by HOMA were obviously higher in both the HDG and the MDG after RBP treatment than that in the DCG, but it was still significantly lower than that in the NCG. However, IRIs of HDG and MDG rats were recognizably smaller than that of DCG rats without RBP treatment.

Table 3 The insulin levels in each rat group after RBP intervention

Group	HDG	MDG	LDG	DCG	NCG
Insulin (ug/ml)	1.114±0.14	1.149±0.15	1.327±0.12 ^{ab}	1.163±0.15	1.237±0.15
Insulin resistance index (IRI)	0.01136±0.00112 ^{ab}	0.01130±0.00135 ^{ab}	0.01746±0.00164 ^b	0.01806±0.00132 ^b	0.00784±0.00093 ^a
Insulin sensitivity index (ISI)	3.91±0.27 ^{ab}	3.94±0.32 ^{ab}	2.55±0.16 ^b	2.46±0.26 ^b	5.67±0.78 ^a

Note: HDG, high-dose group; MDG, middle-dose group; LDG, low-dose group; DCG, diabetic control group; NCG, normal control group; ^a, significantly different at the 0.05 probability compared with DCG in the same row; ^b, significantly different at the 0.05 probability compared with NCG in the same row.

3.4 Oral glucose tolerance test

As shown in Figure 1, the oral glucose tolerance test (OGTT) results suggested that the blood glucose concentration of DCG increased after gastric perfusion of glucose with a peak at 60 min, similar to the LDG. The peaks of HDG and MDG emerged kind of earlier than the DCG's peak, less than 60 min. As to the NCG, the peak appeared after 15 min, much earlier than the other groups. The results implicated that RBP did not delay the increase of postprandial glucose level. However, compared to the DCG control rats, RBP significantly reduced the postprandial glucose peaks of the intervention groups, although the peaks were still recognizably higher than that of NCG. In addition, the areas under the curve of glucose (AUCG) after the glucose gavage in each group were (22.84±4.69) mmol/L (HDG), (20.62±4.27) mmol/L (MDG), (26.94±4.46) mmol/L (LDG), (35.87±7.11) mmol/L (DCG) and (12.40±3.15) mmol/L (NCG). In a word, the OGTT results suggested that, after intervention, RBP could greatly improve glucose tolerance of diabetic rats, but the rats could not ever recover to the normal level.

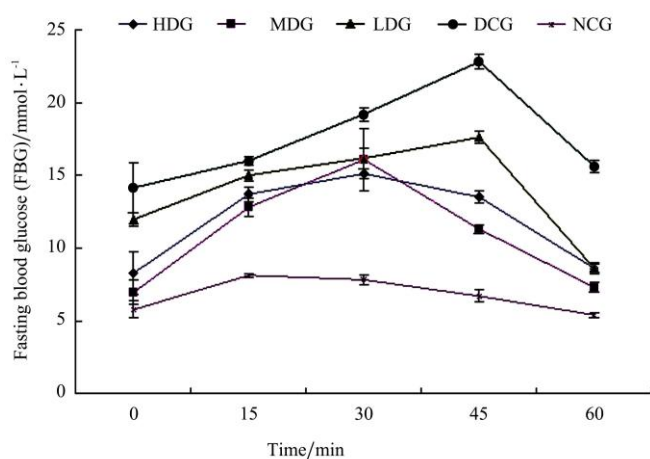


Figure 1 The fasting blood glucose (FBG) is changed in response to different diet and doses. FBG was determined by the oral glucose tolerance test at various time points. The dark blue, pink, yellow, light blue and purple lines indicate high-dose group (HDG), middle-dose group (MDG), low-dose group (LDG), diabetic control group (DCG) and normal control group (NCG), respectively

3.5 Lipid metabolism

Serum CHO and TG concentrations in each rat group

showed different responses after four-week RBP intervention (Table 4). Compared to the NCG, serum CHO levels in the other rat groups were elevated differently. Rat serum CHO levels in the HDG and MDG were significantly lower than that in the DCG. A small and non-significant reduction of CHO was observed in the LDG when using the DCG as the control. Furthermore, CHO concentration in the intervention rat groups was correlated with the amount of RBP. Though CHO levels in the intervention groups were significantly higher than that in the NCG, it appeared that the presence of RBP in these diets negated the CHO-raising potential. This effect may be specific to RS and dietary fiber, probably caused by less weight-gain. In contrast, TG levels in the intervention rat groups surprisingly increased. The rat serum TG levels in the LDG and MDG were significantly higher than that in the DCG.

Table 4 The blood lipid levels in each rat group after intervention

Group	CHO/mmol L ⁻¹	TG/mmol L ⁻¹
HDG	0.887±0.119 ^{ab}	2.103±0.284
MDG	1.252±0.241 ^{ab}	2.360±0.220 ^{ab}
LDG	1.482±0.261 ^b	2.368±0.510 ^{ab}
DCG	1.484±0.245 ^b	2.055±0.348
NCG	0.478±0.112 ^a	2.028±0.308

Note: HDG, high-dose group; MDG, middle-dose group; LDG, low-dose group; DCG, diabetic control group; NCG, normal control group; CHO, Cholesterol; TG, triglycerides; ^a significantly different at the 0.05 probability compared with DCG in the same row; ^b significantly different at the 0.05 probability compared with NCG in the same row.

3.6 SCFA and pH value of colon content

RBP were fermented in the colon, recognized by low colon content pH value and high SCFA amount (Table 5). Compared to two control groups, NCG and DCG, RBP groups had a lower colon pH value and higher SCFA level. Among these RBP groups, the HDG rats showed most significant change. The lower blood glucose might also partially result from the metabolic consequence of gut microflora. As a prebiotic, RBP stimulated the growth or activity of beneficial bacteria in the colon. Although the detailed mechanisms were still unclear, our data indicated that certain actions of banana powder were associated with the fermentation.

Table 5 The short chain fatty acids (SCFA) concentration and pH value of colon content

Group	SCFA ($\mu\text{mol/g}$ wet contents)	pH value
NCG	50.30 \pm 0.59	7.24 \pm 0.40
DCG	32.46 \pm 8.34	7.76 \pm 0.45
HDG	188.50 \pm 46.90	5.67 \pm 0.30
MDG	118.60 \pm 32.41	6.18 \pm 0.74
LDG	61.42 \pm 10.80	6.74 \pm 0.91

4 Discussion

Diabetes is associated with the increasing prevalence of overweight and obesity. Obesity and type-2 diabetes frequently occur together, and statistics showed that 60%-90% of type 2 diabetics are or have been obese^[21,22]. Dietary fiber has been widely used as an effective way to decrease calorie intake and maintain a healthy body weight^[23,24]. The mechanism, by which fiber sources lower body weight, has focused on its effects to reduce food intake and promote satiation or satiety. Some studies supported that increased fiber intake decreases hunger, provides a feeling of fullness and plays a role in the control of energy balance^[25]. On the other hand, body weight reduction is associated with an increase in insulin sensitivity. This association is explained by the reduced demand on insulin production and secretion from the β -cells after losing weight. Moreover, it has been reported that incorporating RS into the diet can decrease body fat storage and increase insulin sensitivity^[26-30] by enhancing the viscosity of the stomach and small intestinal contents^[31]. In this study, the similar result was reported. RBP, which was rich in dietary fiber, could lower weight gain (Table 1) and increase insulin sensitivity (Table 3) in type-2 diabetic rats. This may contribute to the reduction of blood glucose.

In recent years, previous studies have showed the potential impact of RS on glycaemic control and postprandial metabolism^[32-36]. However, opposite results have also been reported^[37,38], which favor the view that RS ingestion has no effect on postprandial glycemia^[39]. These equivocal results might be due to differences in dietary components, texture, and energy content between control diets and dietary fiber diets used. In this study, a significant effect of RBP on FBG in

intervention groups was observed after the intervention, but the FBG level was not able to recover to normal (Table 2). Moreover, it must be highlighted that the rats in the intervention groups did not change their high glucose and high lipid diet during the entire experimentation period. The mechanisms of regulating the blood glucose balance may provide useful information to explain the observed phenotypes. In the present study, the OGTT results showed that RBP did not delay the increase of postprandial glucose level, but it significantly reduced postprandial glucose peak in the intervention groups versus the DCG. However, the peak was still significantly higher than that of NCG (Figure 1).

As reported, both dietary fiber and RS can lower serum total blood lipid effectively. The decreased serum total CHO alone is linked to the reduction of the heart disease risk. In this study, the CHO-lowering effect may be specific to dietary fiber and RS in RBP, which have been repeatedly shown as potent CHO-lowering agents^[40-42]. Alternatively, it may be the consequence of differences in rat weight-gain, or both. Results of feeding trials on rats using RS from Adzuki starch (AS) and tebou starch (TS) suggested that AS and TS had a serum cholesterol-lowering function due to the enhanced levels of hepatic SR-B1 (scavenger receptor class B1) and cholesterol 7-hydroxy-lase mRNA^[43]. The bean starches lowered the levels of serum total cholesterol and VLDL + IDL + LDL cholesterol, increased caecal concentration of SCFA (in particular butyric acid concentration), and augmented faecal neutral sterol excretion.

This study also presented some unexpected results, which are contradictory to our general assumptions. Elevated serum TG levels are considered by some researchers as an independent risk factor in heart disease. Based on the previous studies, TG and CHO levels are usually decreased in those fed RS^[43]. However, our data implicated that this similarity did not hold true for serum TG responses. TG levels of rats that were fed with RBP were not lower than that in the DCG rats after four weeks (Table 4). Contrarily, it was even increased, which may be caused by the high glucose and high lipid diet. Its physiological significance is difficult to assess, but it may

be related, as far as CHO levels are concerned, to the mechanism (s) of regulating hepatic TG storage and excretion. If these observations can be affirmed under more modest RBP intakes, it will suggest that an adaptable proportion of different kinds of dietary fibers may not only add dietary fiber to diet, but also decrease blood lipid levels favourably as we predict.

The effects of RS intake on colon health has been well established^[44]. Because colonic fermentation of dietary fiber increases the SCFA content and decreases the pH value of colon contents, we also measured these characteristics and used them as indicators of banana powder fermentation in the colon. In conclusion, the same result was observed after the gastric perfusion of RBG in the diabetic rats (Table 5). The changes in blood glucose may be correlated with SCFA production. This study was not designed to investigate the biochemical mechanism behind the action, but other groups have reported roles of SCFA and ghrelin participation in this process. Increased SCFA were found in peripheral blood, and their uptake index was augmented into skeletal muscle and adipose tissue after RS administration^[17]. In addition, SCFA augmentation has been shown to potentially increment the expression of functional proteins within the intestine because of its ability to mediate the release of glucagon-like-peptide 2^[45,46]. Another possible mechanism is related to the increased ghrelin hormone peripheral concentration after RS. Ghrelin elevation in plasma has been linked to increased insulin sensitivity in numerous studies^[47].

The dietary fiber and RS as the major components in RBP may contribute to the decreased blood glucose level and increased insulin sensitivity. However, in addition to fiber, RBP contains biologically active components such as antioxidants, minerals and phytoestrogens^[14], and therefore the benefits of RBP may be the result of comprehensive action. RBP supplementation could be an advisable alternative to improve the symptoms of type-2 diabetics.

Further long-term studies on suitable dose of RBP are necessary. Although the rats tolerated three doses used in the present study, a further dose-response study is still

required to test the suitable dose of banana powder to provide insights for the amount of banana powder used in the diet. Moreover, differences in texture and palatability of dietary fiber may also generate different effects in rats. Additional studies are needed to acutely clarify the long-term effect of banana powder in a mixed diet and the main effective components in RBP.

Further long-term studies on mechanisms are recommended based on the following three points: (1) RS in RBP exists not only as a kind of dietary fiber, but also as a kind of starch. Though it is not digested by enzyme hydrolysis, the binding sites between RS and amylases still exist. Thus, it is speculated that competitive inhibition occurs between RS and normal starch, when they appeared simultaneously. (2) Researches showed that there is a regulation network in human bodies, connecting nervous system, endocrine system, metabolic system, micro ecosystem and immune system. In this network, all links are interrelated and interacted^[48]. From the view of microecology, it has been revealed that abnormalities of blood glucose are related to abnormalities of gastrointestinal flora^[49,50]. Therefore, the improvement of intestinal bacterial community structure may be associated with the improvement of the diabetic symptoms. (3) Try to establish and screen one or more novel cell model (s) that can be conveniently used to discover the mechanism (s) about the reduced blood glucose of dietary fiber and RS, or their fermentation products. Moreover, there is a probable relation between dietary and the related enzymes under the glucose metabolism.

In summary, RBP showed positive effect to improve symptoms of type-2 diabetic rats. Our results supported the idea of using banana powder as part of dietary fiber supplementation. The food with proper RBP content helps to keep a normal blood glucose level. Besides, RBP has potential, both from its physiochemical and functional properties, to be applied to processed foods. Use of culls for production of starch will provide a starch that may be competitive in the worldwide starch market, improving banana economics and eliminating a large environmental problem caused by cull bananas.

[References]

- [1] Chockchaisawasdee S, Poosaran N. Production of isomaltooligosaccharides from banana flour. *Journal of the Science and Agriculture*, 2013; 93(1): 180-186.
- [2] Saravanan K, Aradhya S M. Potential nutraceutical food beverage with antioxidant properties from banana plant bio-waste (pseudostem and rhizome). *Food & Function*, 2011; 2(10): 603-610.
- [3] Castillo J L, Aparicio-Trapala M A, Francisco-Luria M U, Cordova-Uscanga R, Rodriguez-Hernandez A, Mendez J D, et al. Effects of native banana starch supplementation on body weight and insulin sensitivity in obese type 2 diabetics. *International Journal of Environmental Research and Public Health*, 2010; 7(5): 1953-1962.
- [4] Aparicio-Saguilan A, Sayago-Ayerdi S G, Vargas-Torres A, Tovar J, Bello-Perez L A. Slowly digestible cookies prepared from resistant starch-rich lintnerized banana starch. *Journal of Food Composition and Analysis*, 30(3-4): 175-181.
- [5] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes, estimates for the year 2000 and projections for 2030. *Diabetes Care*, 2004; 27(5): 1047-1053.
- [6] Wadden T A, Berkowitz R I, Womble L G, Sarwer D B, Phelan S, Cato R K, et al. Randomized trial of lifestyle modification and pharmacotherapy for obesity. *New England Journal of Medicine*, 2005; 353(20): 2111-2120.
- [7] McKeown N M, Meigs J B, Liu S, Wilson P W, Jacques P F. Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. *American Journal of Clinical Nutrition*, 2002; 76(2): 390-398.
- [8] Liese A D, Roach A K, Sparks K C, Marquart L, Agostino R B Jr, Mayer-Davis E J. Whole-grain intake and insulin sensitivity: the Insulin Resistance Atherosclerosis Study. *American Journal of Clinical Nutrition*, 2003; 78(5): 965-971.
- [9] Parillo M, Riccardi G. Diet composition and the risk of type 2 diabetes: epidemiological and clinical evidence. *British Journal of Nutrition*, 2004; 92(1): 7-19.
- [10] Marlett J A, McBurney M I, Slavin J L. Position of the American Dietetic Association: health implications of dietary fiber. *Journal of the American Dietetic Association*, 2002; 102(7): 993-1000.
- [11] Topping D L, Clifton P M. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews*, 2001; 81(3): 1031-1064.
- [12] Englyst H N, Cummings I H. Digestion of the carbohydrates of banana (*Musa paradisiaca sapientum*) in the human small intestine. *American Journal of Clinical Nutrition*, 1986; 44(1): 42-50.
- [13] Englyst H N, Cummings J H. Digestion of polysaccharides of potato in the small intestine of man. *American Journal of Clinical Nutrition*, 1987; 45(2): 423-431.
- [14] Fuentes-Zaragoza E, Riquelme-Navarrete M J, Sánchez-Zapata E, Pérez-Álvarez, J A. Resistant starch as functional ingredient: a review. *Food Research International*, 2010; 43(4): 931-942.
- [15] Behall K M, Scholfield D J, Hallfrisch J G, Liljeberg-Elmstahl H G. Consumption of both resistant starch and beta-glucan improves postprandial plasma glucose and insulin in women. *Diabetes Care*, 2006; 29(5): 976-981.
- [16] Robertson M D, Bickerton A S, Dennis A L, Vidal H, Frayn K N. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *American Journal of Clinical Nutrition*, 2005; 82(3): 559-567.
- [17] Park O J, Kang N E, Chang M J, Kim W K. Resistant starch supplementation influences blood lipid concentrations and glucose control in overweight subjects. *Journal of Nutritional Science and Vitaminology*, 2004; 50(2): 93-99.
- [18] Pérez-Sánchez E. Efectos del consumo de una formulación que incluye almidón resistente de plátano en la glucemia postprandial de pacientes con diabetes mellitus tipo 2. Tesis de Maestría en Ciencias Alimentarias. Universidad Juárez Autónoma de Tabasco: Villahermosa, Tabasco, Mexico, 2007.
- [19] Sambucetti M E, Zuleta A. Resistant starch in dietary fiber values measured by the AOAC method in different cereals. *Cereal Chemistry*, 1996; 73(6): 759-761.
- [20] Reaven G, Abbasi F, McLaughlin T. Obesity, insulin resistance, and cardiovascular disease. *Recent Progress in Hormone Research*, 2004; 59(1): 207-223.
- [21] Stumvoll M, Goldstein B J, van Haften T W. Type 2 diabetes, principles of pathogenesis and therapy. *Lancet*, 2005; 365(9467): 1333-1346.
- [22] Bray G A. Medical consequences of obesity. *The Journal of Clinical Endocrinology and Metabolism*, 2004; 89(6): 2583-2589.
- [23] Delzenne N M, Cani P D. A place for dietary fibre in the management of the metabolic syndrome. *Current Opinion in Clinical Nutrition and Metabolic Care*, 2005; 8(6): 636-640.
- [24] Slavin J L. Dietary fiber and body weight. *Nutrition*, 2005; 21(3): 411-418.
- [25] Slavin J L, Green H. Fibre and satiety. *Food and Nutrition Bulletin*, 2007; 32: 32-42.
- [26] Brown I L. Applications and uses of resistant starch.

- Journal of AOAC International, 2004; 87(3): 727-732.
- [27] Higgins J A. Resistant starch: metabolic effects and potential health benefits. *Journal of AOAC International*, 2004, 87(3): 761-768.
- [28] Deckere E A, Kloots W J, van Amelsvoort J M. Resistant starch decreases serum total cholesterol and triacylglycerol concentrations in rats. *Journal of Nutrition*, 1993; 123(12): 2142-2151.
- [29] Cassidy A, Bingham S A, Cummings J H. Starch intake and colorectal cancer risk: an international comparison. *British Journal of Cancer*, 1994; 69(5): 937-942.
- [30] Udagawa H, Kitaoka C, Sakamoto T, Kobayashi-Hattori K, Oishi Y, Arai S, et al. Serum cholesterol-decreasing effect of heat-moisture-treated high-amylose cornstarch in cholesterol-loaded rats. *Bioscience, Biotechnology, and Biochemistry*, 2008; 72(3): 880-884.
- [31] Cameron-Smith D, Collier G R, O'Dea K. Effect of soluble dietary fibre on the viscosity of the gastrointestinal contents and the acute glycaemic response in the rat. *British Journal of Nutrition*, 1994; 71: 563-571.
- [32] Sajilata M G, Singhal R S, Kulkarni P R. Resistant starch: A review. *Comprehensive Reviews in Food Science and Food Safety*, 2006; 5(1):1-17.
- [33] Han J A, BeMiller J N. Preparation and physical characteristics of slowly digesting modified food starches. *Carbohydrate Polymers*, 2006; 25(7): 1-9.
- [34] Mun S H, Shin M. Mild hydrolysis of resistant starch from maize. *Food Chemistry*, 2006; 96(5): 115-121.
- [35] Shamai K, Bianco-Peled H, Shimoni E. Polymorphism of resistant starch type III. *Carbohydrate Polymers*, 2003; 54(11): 363-369.
- [36] Frei M, Siddhuraju P, Becker K. Studies on the in vitro starch digestibility and the glycemic index of six different indigenous rice cultivars from the philippines. *Food Chemistry*, 2003; 83(11): 395-402.
- [37] Roos N, Heijnen M L, de Graaf C, Woestenenk G, Hobbel E. Resistant starch has little effect on appetite, food intake and insulin secretion of healthy young men. *European Journal of Clinical Nutrition*, 1995; 49(7): 532-541.
- [38] Udagawa H, Kitaoka C, Sakamoto T, Kobayashi-Hattori K, Oishi Y, Arai S, et al. Increase of serum cholesterol levels by heat-moisture-treated high-amylose cornstarch in rats fed a high-cholesterol diet. *Lipids*, 2008; 43(8): 695-702.
- [39] Jenkins D J, Kendall C W, Axelsen M, Augustin L S, Vuksan V. Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease. *Current Opinion in Lipidology*, 2000; 11(1): 49-56.
- [40] Pilch S M. Physiological effects and health consequences of dietary fibre, life science research office. Federation of American Societies for Experimental Biology, Bethesda, Maryland. Contract Number FDA 223-84-2059, pp. 162-168, 1987.
- [41] Ranhotra G S, Gelroth J A, Glaser B K. Effect of pectin-wheat bran blends on rat blood lipid and fecal responses and on muffin quality. *Cereal Chemistry*, 1993; 70(5): 530-534.
- [42] Anderson J W, Jones A E, Riddell-Mason S. Ten different dietary fibers have significantly different effects on serum and liver lipids of cholesterol-fed rats. *Journal of Nutrition*, 1994; 124(1): 78-83.
- [43] Han K H, Fukushima M, Kato T, Kojima M, Ohba K, Shimada K, et al. Enzyme-resistant fractions of beans lowered serum cholesterol and increased sterol excretions and hepatic mRNA levels in rats. *Lipids*, 2003; 38(9): 919-924.
- [44] Willowski I, Rechkemmer G, Pool-Zobel B L. Protective role of probiotics and prebiotics in colon cancer. *American Journal of Clinical Nutrition*, 2001; 73(Suppl 2): 451S-455S.
- [45] Reilly K J, Frankel W L, Bain A M, Rombeau J L. Colonic short chain fatty acids mediate jejunal growth by increasing gastrin. *Gut*, 1995; 37(1): 81-86.
- [46] Tappenden K A, McBurney M I. Systemic short-chain fatty acids rapidly alter gastrointestinal structure, function, and expression of early response genes. *Digestive Diseases and Sciences*, 1998; 43(7): 1526-1536.
- [47] Poykko S, Ukkola O, Kauma H, Savolainen M J, Kesaniemi Y A. Ghrelin Arg51Gln mutation is a risk factor for Type 2 diabetes and hypertension in a random sample of middle-aged subjects. *Diabetologia* 2003; 46(4): 455-458.
- [48] Cryer P E, Polonsky K S. Glucose homeostasis and hypoglycemia. *Williams Textbook of Endocrinology*. Harcovert publishers Limited, 9th ed, 1998.
- [49] Tanaka J, Fukuda Y S, Hori K, Tomita T, Ohkusa T, Matsumoto T, et al. Influence of antimicrobial treatment for helicobacter pylori infection on the intestinal microflora in Japanese macaques. *Journal of Medical Microbiology*, 2005; 54(3): 309-314.
- [50] Zoetendal E, Akkerns A, de Vos W. Temperature gradient gel electrophoresis analysis of 16S RNA from human fecal samples reveals stable and host specific communities of active bacteria. *Applied and Environmental Microbiology*, 1998; 64(10): 3854-3859.