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Effects of Incorporating green banana flour on snack bar glycemic response in normal volunteers

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Food with lower glycemic index is related with decreased risk of several chronic diseases, such as heart disease and Type-2 diabetes. This work was aimed to produce low glycemic index snack bars by partial substitution with green banana flour (GBF) for rolled oats at 15%. Snack bar without GBF represented the control. The glycemic index and glycemic load of snack bars with GBF (15%) or without GBF were determined in healthy volunteers. Eight subjects with average age (\pm standard deviation) of 25 ± 1.49 years and average body mass index (\pm standard deviation) of 22.34 ± 2.52 , were tested on seven occasions for non-consecutive days. Subjects were required to consume 50 g of available carbohydrate as a portion of snack bars or a glucose drink (reference) after 10-12 hours overnight fast. Blood glucose was taken for 120 minutes at 15-minute interval in the first hour and 30-minute interval in the second hour after consumption. Incremental area under the curve for glucose response was calculated for each snack bar and compared with that of glucose drink to determine the glycemic index. Results showed that the glycemic index and glycemic load for snack bar which contained GBF (31.01 ± 16.94 ; 15.51 ± 8.47 , respectively) were lower than that of control (31.71 ± 6.96 ; 15.85 ± 3.47 , respectively). These research findings could be a useful platform to develop low glycemic index (< 55) snack bars as an alternative snack for individuals in maintaining a desirable blood glucose response.

Keywords: Glycemic response, green banana flour, *in vivo*, snack bar

INTRODUCTION

Snack bars have increased in popularity and demand from consumers, especially people in developed countries because they are convenient, ready-to-eat, portable, and come with various properties, including nutrition and functional aspects (Miller et al. 2006; Onfry et al. 2014; Ho et al. 2016). However, most snack bars sold in the market have high amount of sugar, thus, high in glycemic index which cause a sharp increase in blood sugar for energy supplied, and thus lead to an increased risk for obesity, Type-2 diabetes and

other diseases associated with aging (Dahri et al. 2017). According to Chepulis and Francis (2013), the costs of global financial spent for annually treatment of these global diseases were more than hundreds of billions of dollars. This situation indicating that an immediate action needs to be taken. The possible use of alternative, lower glycemic index of snack foods, including snack bar, is therefore one way to address this need.

Glycemic index is defined as the incremental area under the curve of blood glucose response of an available carbohydrate portion (50 g) for a test

food. It is presented as percentage of the response to the same amount of carbohydrate from a reference food (i.e., glucose solution or white bread) taken by the same subject (WHO/FAO, 1998; Sabeetha et al. 2018). The glycemic index is a way to classify carbohydrates in food according to their effects in raising the blood glucose level (Lunn and Buttriss, 2007). Therefore, the food selection is crucial as it can influence the digestion rate of carbohydrates and result in the blood glucose level response (Schulze et al. 2004). Higher glycemic index values show a greater rise in blood glucose after ingestion and vice versa (Nansel et al. 2006; Supparmaniam et al. 2019). Consuming food such as snack bars with low glycemic index is recommended for people with diabetes to regulate their diet (Ferrer-Mairal et al. 2012; Jariyah et al. 2018). Furthermore, according to Supparmaniam et al. (2019), foods with lower value of glycemic index may help to control satiety mechanisms and therefore, regulates body weight.

Only a few research studies on the production of low glycemic index snack bars were published. For example, the low glycemic index (34.8) granola bar was successfully produced by partially substituting wheat flour with pulse ingredients without changing the sensory of products (Fujiwara et al. 2016). A study by Zamora-Gasga et al. (2014) showed that agave fiber-containing snack bar has glycemic index of 77.26. This high glycemic index food is not suitable for patients who are suffering from Type-2 diabetes disease. Therefore, a previous study had succeeded in developing a low glycemic index snack bar which was formulated with green banana flour. It was found that the developed snack bar had an estimated glycemic index (eGI) value of approximately 47.6 – 46.38 (Ho et al. 2018). Therefore, all produced green banana flour containing snack bars are classified as low glycemic foods (< 55). It has potential to reduce the spike in blood glucose that occurs after consuming a snack bar. However, the glycemic index value was obtained through computation of results obtained from an in vitro enzymatic assay.

According to Brand-Miller and Holt (2004), the in vitro assays may be applicable for the initial stage of food product development, whereby, factors that affect the rate of carbohydrate digestion in food is investigated. However, these assays are not able to detect neither the viscosity effect on absorption rate of the products of digestion nor the profound effect of differences in gastric emptying that is caused by volume,

concentration, acidity, and osmolality of sugars (Brand-Miller and Holt, 2004). Therefore, the in vitro measure will misrepresent the true glycemic effect of carbohydrates in food; hence, glycemic index values obtained via in vitro method cannot be used for food labelling purposes because incorrect labeling is potentially harmful to someone with diabetes. Therefore, access to accurate information about the glycemic index value of food through in vivo assay is important for food labeling purposes to assist consumers in making informed dietary choices. Therefore, the present work in glycemic index measurement via in vivo assay needs to be carried out to confirm that the produced snack bars are of low glycemic index before it is transferred to interested industry partners for commercialization of green banana snack bars.

MATERIALS AND METHODS

Materials

Ingredients such as rolled oats, skimmed milk powder, goji berries, pumpkin seeds, and sorbitol were purchased at Pantai Timor (Jerteh) Supermarket, Jerteh, in Terengganu. Unripe green banana was obtained from a local wet market, in Jerteh, Terengganu.

Processing of green banana flour

Unripe green bananas (*Musa acuminata* x *balbisiiana* ABB, variety: Awak) without yellow/brown spots were selected for production of green banana flour (GBF). The banana skin was manually peeled with a clean knife. Then the pulps were cut into small pieces prior to soaking in sodium metabisulfite solution (concentration: 1:1,000). The pulp was sliced by using a fruit slicer (Santos, Vegetable Slicer 48, Lyon, France) before drying overnight in a ventilated dryer at 60 °C (Tech-Lab, FDD-720, Selangor, Malaysia). The dried slices were ground by using a laboratory mill (Panasonic, MX-801S, Selangor, Malaysia) to produce fine powder.

Study design

The standard glycemic index procedure was followed according to Wolever et al. (1991). Eight healthy volunteers were selected for the glycemic index determination. Ethical approval was obtained from UniSZA Human Research Ethics Committee (UHREC) (UHREC Code: UniSZA/UHREC/2019/112). The study undertaken was single-blinded and all samples, including glucose drink were randomized throughout the

seven testing sessions (i.e., three testing sessions for reference food and two testing sessions for each test food).

Subjects

All subjects (8 females) were asked to complete the informed consent document prior testing. The average age of the volunteer was 25 years (range 24-29 years). Height and weight of volunteers were measured by a trained research officer and used to calculate the body mass index (BMI). Average BMI of the volunteer was 22.34 kg/m² (range 18.5 – 25.00 kg/m²).

The study excluded subjects who were on antibiotic medication, pregnant, lactating, smokers, subjects with gastrointestinal disease such as coeliac disease, lactose intolerant, allergic to gluten, on dietary restrictions, vegetarian, subjects on regular use of dietary supplements, subjects with a significant changes in body weight for the past one year (\pm 4 kg), known chronic illnesses, such as diabetes or impaired glucose tolerance, and being prescribed with medication known to affect the blood glucose.

Test Meals

Reference food

The reference food was beverage prepared from 50 g of D-glucose anhydrous (Glucolin, Samutprakam, Thailand). The D-glucose solution was prepared in 400 mL of water.

Test food

Two types of snack bars were prepared in this study. Toasted rolled oats was partially replaced by GBF at 15% level to prepare SBF15 (Table 1). The percentage of substitution was selected based on the preliminary study results obtained in preference test which received the highest score in 'overall acceptance' for snack bar as rated by sensory panellists (Dahri et al. 2017). Snack bar prepared without GBF (SBF0) was used as a control. All the dry ingredients were mixed by using a blender (Waring Commercial Blender, 7011HS, Osaka, Japan). Wet ingredient (i.e., sorbitol syrup) was then added into the mixture and blended until homogenous. The mixture was shaped into cuboids (3 cm x 9 cm x 0.8 cm) prior to drying by using a ventilated dryer (Tech-Lab, FDD-720, Selangor, Malaysia) at 40 °C for 3h. Each serving of snack bar was prepared to contain 50 g of available carbohydrates.

Calculation of portion size of samples

Available carbohydrates were calculated by difference [Available carbohydrates = total carbohydrates – total dietary fibers in 100 g of food] (BeMiller and Low, 1998). The subjects had to consume 97.77 g and 180.25 g of SBF0 and SBF15 snack bar, respectively, within 15 minutes. The information on available carbohydrates in 100 g of samples is presented in Table 2.

Table 1: Formulations of snack bars preparation

Ingredients	Types of Snack Bar ¹	
	SBF0	SBF15
Toasted rolled oats (g)	30.0	25.5
Green banana flour (g)	-	4.5
Skimmed milk powder (g)	20.0	20.0
Goji berry (g)	20.0	20.0
Pumpkin seeds (g)	10.0	10.0
Sorbitol (mL)	20.0	20.0

¹SBF0: snack bar containing green banana flour; SBF15: snack bar without green banana flour.

Table 2: The information of samples¹

	Samples ¹		
	Reference (D-glucose)	SBF0	SBF15
Available carbohydrate (% g, 100 g)	99.40	51.14	46.19
Weight required to deliver 50 g available carbohydrate (g)	50.30	97.77	108.25

¹SBF0: snack bar containing green banana flour; SBF15: snack bar without green banana flour.

Blood glucose testing

Subjects were studied on seven (7) different occasions in the morning after 10 – 12 h overnight fasting. No restrictions were placed on food intake prior to test. On three different occasions, the subjects consumed 50 g of glucose (reference food) dissolved in 400 mL of water. Reference food was repeated three times to reduce subject variability (Wolever et al. 2003). The same subject repeated the procedure for test food (SBF15 and SBF0). The test meal which contained 50 g available carbohydrates was given to the subjects with 250 mL drinking water. Each subject was advised to consume the meal between 10 and 15 minutes. Blood samples (1.0 μ L) were obtained by finger-prick with a lancet (Cofoe Yili, Sinocare, China) and were collected into a test strip (Cofoe Yili, Sinocare, China) via a blood absorbing port.

The blood was collected at 0 minutes before food was consumed and after 15, 30, 45, 60, 90, and 120 minutes after meal. Then, the test strip was inserted into a portable glucometer (Cofee Yili, Sinocare, China) for blood glucose determination (Wolever et al. 1991). The test meal was repeated twice to obtain the mean value of blood glucose level.

Computation of glycemic index

Glycemic index was calculated from the incremental area under the curve (iAUC) of the blood glucose response curve of a 50 g available carbohydrates portion of the test food and it was expressed as a percentage of the response to 50 g of available carbohydrates from the reference food (D-glucose) consumed by the same subject: $[(\text{iAUC of 50 g available carbohydrates test food} / \text{iAUC of 50 g available carbohydrate of reference food}) \times 100]$. The area under the curve was computed by using Microsoft Excel 2010 software.

Calculation of glycemic load

Glycemic load was determined by calculation: $[(\text{glycemic index value} \times \text{grams of carbohydrate}) / \text{servings}] / 100$ (Miller et al. 2006).

Statistical analysis

Statistical analysis was conducted by using statistical package for the social science (SPSS) Version 20.0 software (SPSS Inc., Chicago, IL, USA). The present recorded results were represented as the mean values \pm standard deviation. The data were subjected to one-way analysis of variance (ANOVA). The significant differences among test meals for individual's mean blood glucose concentrations at each time was determined by Tukey's HSD test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Blood glucose response of snack bars

The average blood glucose responses after the ingestion of reference food (glucose) and two test food (SBF0 and SBF15) at 0, 15, 30, 45, 60, 90, and 120 minutes are shown in Figure 1. In general, blood glucose will response after ingestion of reference food (anhydrous glucose). Results indicated that the glycemic response curve for all snack bars was shown by the fluctuations of the glucose absorption in blood. All snack bars showed similar patterns in producing the blood glucose responses in healthy volunteers, whereby, all tested snack bars

demonstrated lower blood glucose responses two-hour postprandial as compared to blood glucose responses to a reference food (i.e., 50 g glucose load) for the same subject. However, the blood glucose responses at various time intervals were due to intake of snack bars that contained green banana flour was lower than the control snack bar (snack bar without green banana flour).

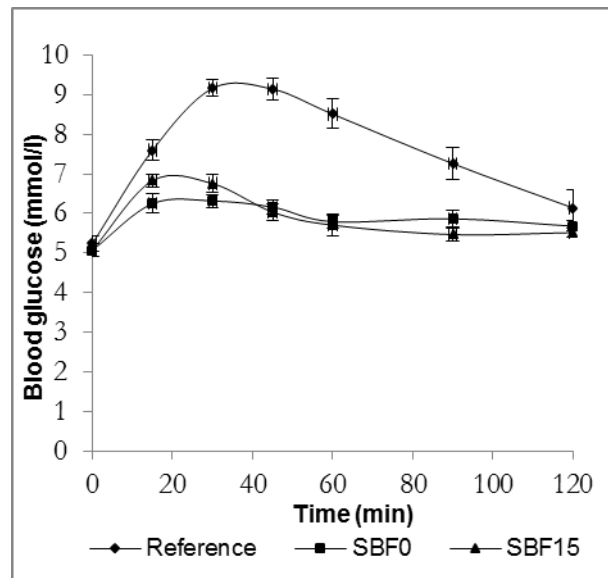


Figure 1: Average incremental blood glucose responses of the two snack bar samples versus a reference food in healthy subjects (n = 8).

SBF0: snack bar containing green banana flour; SBF15: snack bar without green banana flour.

According to Ludwig (2002), blood glucose response to the ingestion of carbohydrate in a food varied greatly, depending on several factors such as the molecular structure of carbohydrates, fiber content (i.e., soluble dietary fiber and insoluble dietary fiber), and processing techniques. Some indigestible carbohydrates such as resistant starch was shown to behave more likely to dietary fibers that were linked with improvements in glycemic control (Nugent, 2005). The amylose contents present in starches could affect the digestion rate (Shanita et al. 2011). The higher amylose content in green banana flour (23.1 – 32.05%) (Reddy et al. 2015) as compared to oats (22.7 – 22.9%) (Hoover and Senanayake, 1996) was the main contributor to slow digestion starch (Nugraheni et al. 2018) of SBF15. Furthermore, resistant starch in food (e.g., Type-2

resistant starch in green bananas) caused the starch not to be hydrolyzed by the digestive enzymes (i.e., α -amylase); hence, slowed down glucose absorption in the small intestine (Nugraheni et al. 2018).

In addition, fiber rich foods are capable of reducing the glycemic response; consequently, minimizing the need of insulin and playing a vital role in delaying the growth rate of bad microbial in the large intestine (Ho et al. 2015). It was reported that dietary fiber may delay the postprandial blood glucose responses, whereby, the rate of carbohydrate absorption is reduced due to a viscous gel formation in the small intestine (Ali et al. 2010). It corresponded with a research, which reported that the large intake of dietary fiber was related to better glycemic index control (Fujii et al. 2013). The present obtained results were in agreement with previous findings, which revealed that the formulating of green banana flour in rolled oats snack bars had significantly increased the functional aspects, such as dietary fiber (i.e., 4.31% –10.07% of insoluble dietary fibers, 4.35% – 4.58% of soluble dietary fibers, 8.66% – 14.65% of total dietary fiber, and 6.22– 7.49% of resistant starch) of the snack bar (Ho et al. 2018). According to Lestari et al. (2020), non-digestible starch (i.e., resistant starch), non-digestible oligosaccharide, and dietary fibers are not digested, and thus the glycemic response is low.

Glycemic index and glycemic load of snack bars

The glycemic index and glycemic load profile of snack bars are tabulated in Table 3. Analysis of variance results of the data glycemic index value indicated that the substitution of green banana flour for rolled oats in snack bar making (SBF15) showed almost similar glycemic index values. However, snack bars with green banana flour (31.0) had lower value of glycemic index than the control snack bar (31.70) (Table 3).

Table 3: Glycemic index and glycemic load of reference food (D-glucose) and test food

	Samples ¹		
	Reference (D-glucose)	SBF0	SBF15
Glycemic index	100.0 ^a	31.7 ^b	31.0 ^b
Glycemic load	50.0 ^a	15.9 ^b	15.5 ^b

Data are presented as mean (n = 8). Mean values in the same row with same superscript lower letters are

not significantly different at $P > 0.05$.

¹SBF0: snack bar containing green banana flour; SBF15: snack bar without green banana flour

Low and high glycemic index foods can be distinguished by the speed of digestion and absorption of glucose and fluctuation levels in blood (Nugraheni et al. 2018). According to Manullang et al. (2020), there are three classification of glycemic index in food: <55 indicates low glycemic index; 55–70 indicates intermediate glycemic index, and; >70 indicates high glycemic index. Therefore, snack bars processed in the present study could be categorized as low glycemic index food since the glycemic value was below 55. This means that the rates of carbohydrates digestion and stomach emptying after consumption of these snack bars were slower. This caused the suspension of food (chyme) to reach the small intestine slower, then a delayed digestion and poor glucose absorption in the small bowel. Finally, fluctuations in blood glucose levels were relatively lower (Manullang et al. 2020).

The differences in glycemic index values may be due to several factors such as different ingredients (i.e., green banana flour) used in the preparation of these snack bars. Green banana contains high amount of resistant starch (i.e., Type-2 resistant starch) and comparable behavior to fiber, which is highly resistant to the digestive system enzyme (Englyst et al. 1992). Therefore, it is expected to improve the overall blood glucose control in the small intestine by reducing the glucose response. These obtained results are in accordance with a study done by Saifullah et al. (2009), in which yellow noodles that were incorporated with green banana flour presented lower glycemic index than the yellow noodle without green banana flour (control). According to Bernatal et al. (2019), food with low glycemic index can improve metabolic control in adults with Type-2 diabetes mellitus. However, further research are needed to investigate the effects of snack bars prepared from green banana flour on glycemic index in diabetic patients and obese persons. However, based on this current result, the lower glycemic index response for test foods than a reference food (glucose) suggested that all the snack bar prepared with or without green banana flour may also be a good choice of snack for them.

Glycemic index provides information regarding the rate of carbohydrate digestion and blood glucose absorption, but it does not

represent the carbohydrates amount and the impact that certain foods have on blood glucose level (Manullang et al. 2020). The important of low glycemic index food intake is to reduce glycemic load. Glycemic load is a product of glycemic index and amount of carbohydrate ingested, indicating availability of sugar (glucose) for energy or storage following a carbohydrate containing meal (Jariyah et al. 2018). According to Lestari et al. (2020), glycemic load is a metric used for weight management as well as to control diabetes. Therefore, food with high glycemic load could result in an increased risk of obesity and diabetes. Snack bar with incorporation of 15% green banana flour (SBF15) had lower glycemic load value than the snack bar without green banana flour (SBF0) (Table 3). According to Manullang et al. (2020), glycemic load of food can be classified into three categories: <10 as low glycemic load; 11-19 as medium glycemic load; and >20 as high glycemic load. Therefore, all the produced snack bars had medium glycemic load (15.51–15.85). This indicated that there was a small amount of glucose in the snack bar available for energy use or storage after consumption. However, it only causes slow elevation of blood glucose levels.

The glycemic index of a food can be decreased by decreasing the glycemic index or eliminating the quantity of carbohydrates from food. This is due to the different types of carbohydrate in the ingredients, resulting in different blood glucose responses (Lestari et al. 2020). Snack bars which were prepared from green banana flour had blood glucose lowering effect. Green banana flour had a low glycemic index of 40 (Foster-Powell et al. 2002). In addition, a study completed by Okafor and Ugwu (2013) demonstrated that banana, particularly plantain and green banana had high slowly digestible starch (a low glycemic index value). This was attributed to the presence of high amount of resistant starch (i.e., type-2 resistant starch) and dietary fibers of unripe plantain (Okafor and Ugwu, 2013). Besides, beta glucan (β -glucan), which is a soluble fiber, is a major component of oats and could form a viscous solution in the digestive system. Its viscous solution is very effective in reducing postprandial glucose and insulin responses (Mäkeläinen et al. 2007). Several studies have shown that food samples containing oats β -glucan had lower glycemic index as compared with samples without oats β -glucan (Mäkeläinen et al. 2007; Choo and Abdul Aziz, 2010). In addition, β -glucan was reported to contribute in enhancing satiety

(Hetherington et al. 2013).

Another ingredient in snack bars which have a lowering effect is goji berry. Although goji berry has a sweet taste, it has a glycemic index of only 29 (Tibet Authentic, 2012). Kulczyński and Gramza-Michałowska (2016) reported that goji berry-prepared food for alloxan-induced diabetic rabbits demonstrated to significantly decrease the concentration of blood glucose in tested rabbits, after treatment for approximately 10 days. Besides, snack bars were added with sorbitol, a sugar alcohol or referred as polyols, which is often used as a bulking agent for low calorie food and acts as a good humectant as it holds moisture and preserves foods from drying out (Ho and Pulsawat, 2020). Sorbitol has very low glycemic index, as little as 10. Food containing sorbitol can be used by diabetic patients to manage their blood glucose levels (Deis and Kearsley, 2012).

CONCLUSION

All snack bars have a glycemic index value of below 55 and can be classified as low glycemic index food group. However, snack bars containing green banana flour (SBF15) showed a lower glycemic index value than the control snack bar (SBF0). The research findings obtained in this study showed that green banana flour could be beneficial as functional ingredient for the development of low glycemic index (<55) snack bars. The developed snack bars can serve as an alternative snack for diabetic patients and weight management people. These produced snack bars are expected to control the blood glucose levels among diabetic patients.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Lee-Hoon Ho wrote the manuscript, Mazaitul Akma Suhaimi performed the experiment, and all authors read and approved the final version.

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