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FULL PAPER

The pre-treatment effect of *Kuini*'s kernel powder on starch digestibility and estimated glycaemic index (eGI)

[1]Noor Fadzlina, I.Z.A., [1]Tun Norbrillinda, M., [2]Hadijah, H. and [1]Nur Intan Farina, S.

[1]*Food Designing, Processing and Packaging Programme, Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), G.P.O Box 12301, 50774 Kuala Lumpur, Malaysia*

[2]*Food Nutrition and Safety Programme, Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), G.P.O Box 12301, 50774 Kuala Lumpur, Malaysia*

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Abstract

Mangifera odorata or *Kuini* is an indigenous fruit in Malaysia. The work aimed to study the effect of different pre-treatment of *Kuini's* kernel powder on their starch digestibility and estimated glycaemic index (eGI). The kernel powder was processed with two different pre-treatments which are steaming and blanching. Steaming treatment was applied for 5 mins (S5), 10 mins (S10) and 15 mins (S15). While blanching treatment was applied for 10 mins (B10). Control kernel powder (CP) was processed without any pre-treatment. Starch digestibility, estimated glycaemic index and total starch were analysed for all samples. CP has an eGI of 60 that can be classified as medium GI food. Both pre-treatment processes decreased the digestible starch component which resulted in a decrease in the glycaemic index values. This finding indicated that heat-treated *Kuini's* kernel powders can be classified as low glycaemic food with eGI below 55. Low-GI and medium-GI foods are slowly digested, absorbed and metabolized resulting in stable fluctuation in blood glucose levels.

1. Introduction

Kuini is one of the locally indigenous plants in Malaysia. It belongs to the *Anacardiaceae* family and has a very similar appearance to the *Mangifera indica* (mango), *Mangifera foetida* (‘*bacang’*), *Mangifera pajang* (Bambangan) and *Mangifera caesia*. Kiew, (2002) reported that *Kuini* is a hybrid between *Mangifera indica* (mango) and *Mangifera foetida* (‘*bacang’*). It has a strong aroma with a vibrant orange-yellow colour of skin and flesh when it is ripe. *Kuini* is a seasonal fruit that is highly perishable due to its soft and fragile texture. It starts deteriorating once fully ripened. To avoid fruit glut, *Kuini* will be converted to a semi-processed product such as puree, pulp, and nectar. However, *Kuini*’s processing industry led to excessive by -products such as peel and kernel (Figure 1). This by-product has nutritional value almost the same as the flesh and could be processed into a high-value functional ingredient (Ayala-Zavala *et al*., 2011). This fruit and vegetable by-product is rich in bioactive compounds and dietary fibre as shown in Table 1. The dried kernel can be processed into a powder form and incorporated into various foods as a functional food additive.

Figure 1. *Kuini*’s kernel

According to Itam *et al*. (2012), thermal treatments like baking, roasting, frying and boiling can affect the starch digestibility and glycaemic index of starchy food. No previous studies have investigated steaming and blanching treatments. However not only does the thermal processing method affects the starch digestibility and GI of foods, but the starch type, molecular structure and food composition should also be taken into consideration. Furthermore, the GI value of a food cannot be measured by simply looking at the composition of the food, instead, it requires more precise methods such as *in vivo* or *in vitro*. Generally, the *in vivo* method involves humans as a subject matter where the

*Corresponding author.
Email: *fadzlina@mardi.gov.my*

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Table 1. Nutritional value of different parts in fruits

Fruit	Part of the fruit	Phenolics (mg/100 g)	Ascorbic acid (mg/100 g)	Carotenoids (µg/100 g)	Fibre (mg/100 g)	References
Jackfruit	Seed	2770*	-	1910	-	Chandrika *et al.* (2005); Soong and Barlow (2004)
	Pulp	90*	8.0-10.0**	4530**	1600**	
	Peel	-	-	-	-	
Mango	Seed	11,700*	-	-	-	Soong and Barlow (2004)
	Pulp	240*	19.7**	4530**	1000**	
	Peel	7000**	-	-	28,100*	
Avocado	Seed	5160**	-	630*	-	Leong and Shui (2002); Wang *et al*. (2010)
	Pulp	490**	9**	590**	5000**	
	Peel	1260**	-	1520*	-	

-: No result was reported, *Dry weight, **Fresh weight

cost will be expensive, while the *in vitro* method involves analysis techniques in the laboratory at a lower cost. The digestibility of starch is influenced by numerous factors such as digestion rate, food type, food components (protein content, carbohydrates and fats), food preparation methods (boiled, grilled and fried), amylose content, molecular structure (Bao *et al.,* 2018) and physiological effects (hormonal and enzyme reactions). It relies a lot on the samples/subjects used in each investigation. Goni *et al.* (1997) have established an analysis to measure the hydrolysis levels of starchy foods. The data obtained is used to estimate the value of the glycaemic index (eGI) in food.

Glycaemic index (GI) is a system that ranks carbohydrate food from 0 to 100 according to how much they cause the blood sugar to rise after ingestion. According to Brand-Miller *et al*. (1996), foods may be divided into three groups: food with low GI (55 or less), food with moderate GI (56-69) and food with a high GI (70 and more). Glucose from high GI value food will be absorbed into the bloodstream and blood glucose levels will increase rapidly. A stable blood glucose level is a good source of energy for the brain and muscles. Apart from that, low blood glucose levels or hypoglycemia can cause tiredness or sometimes loss of consciousness. A sudden decrease in blood glucose levels could happen especially when glucose levels in the blood increase rapidly before that. Foods with moderate/low GI values will take a slower time to be converted to glucose and absorbed into the bloodstream gradually and steadily (Goni *et al*., 1997).

Therefore, the objective of this study was to evaluate the effect of different pre-treatment such as steaming and blanching on starch digestibility and estimated glycaemic index of *Kuini*'s kernel powder.

2. Materials and methods

2.1 Materials

Kuini fruits were obtained from MARDI Sintok, Kedah, Malaysia and were fully ripened. Enzymes such as pancreatic α-amylase (Sigma NoA-3176, 28 IU/mg), pepsin (Merck No. 7190,2000 FIT-µ/g) and amyloglucosidase (Boehringer No. 102857,15 IU/mg) and glucose-peroxidase kit (SIGMA GAGO20) were purchased from Sigma Aldrich. All chemicals used were of analytical grade.

2.2 Kuini's kernel powder

Fruits were washed for 15 mins using a washer to remove dirt and soil at the surface. Then, clean fruits were placed on a tray in the steam cooker covered with a lid and steamed for 5, 10 and 15 mins. Another batch of *Kuini* fruit was blanched in hot water at 90°C for 10 mins. Fresh *Kuini* fruits without any treatment were used as a control for this study. After pre-treatment, fruits were cooled in ice rapidly. After cooled, all kernels were removed, thinly sliced and oven-dried at 60°C until reached moisture content below 5% followed by a grinding process to produce a powder. All samples were evaluated for their starch digestibility and estimated glycaemic index according to Goni *et al*. (1997).

2.3 In-vitro glycaemic index analysis

The in-vitro glycaemic index analysis was determined according to the method established by Goni *et al.* (1997) and Nednapis (2013).

A total of 50 mg of food sample was weighed and cooked with distilled water at a boiling temperature of 100°C for 15 min using the induction kitchen. It was then added with a pepsin solution at 40°C (60 mins, pH 1.5) for protein hydrolysis. The sample solution was then added with a Tris-maleate buffer solution and the pH is adjusted to 6.9. Starch hydrolysis begins with the addition of 5 mL of Tris-maleate buffer solution containing 2.6 IU of a-amylase solution (Sigma–Aldrich) and incubated at 37°C. At an interval of 30 mins, starting from 0 to 3 hrs, 1 mL of the sample was removed and inserted into another tube. The enzyme α-amylase was de -activated immediately with the boiling process at a

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temperature of 100°C for 5 mins. The hydrolysed starch was fully hydrolysed to glucose by adding 60 µL amyloglucosidase (23,000 units/g solid) and incubated at 60ºC for 45 mins. The glucose released was quantified using GAGO20 reagent assay kit. Starch hydrolysis is measured as mg glucose × 0.9 (conversion factor from glucose to starch). Starch hydrolysis was expressed as a percentage of the number of starches hydrolysed at different times. Each sample was analysed in triplicates. The non-linear model developed by Goni *et al*. (1997) was used to describe the kinetic hydrolysis of starch. The curve indicated that the starch hydrolysis followed a first -order reaction equation; the reaction equilibrium concentration (C∞) and the first-order reaction dynamic constant (k) was obtained through the calculation of the regression equation. The area under the hydrolysis curve (AUC) was calculated with the formula of Goni *et al*. (1997) as per equation (1) below:

$$\mathrm{AUC} = C\infty\left(t_f - t_0\right) - \frac{(C\infty/\mathrm{k})}{[1 - exp - k\left(t_f - t_0\right)]} \quad (1)$$

where C∞ corresponds to the concentration at equilibrium (t180), t_f is the final time (180 mins), t_0 is the initial time (0 min), and k is the kinetic constant.

The hydrolysis index (HI) of each sample was the ratio (%) of the area under the hydrolysis curves between each sample and that of the glucose solution. Therefore, from the HI obtained in vitro we estimated the predicted GI using equation (2) established in the mentioned study by Goni *et al*. (1997).

$$\mathrm{eGI} = 39.71 + 0.549\ \mathrm{HI}. \quad (2)$$

2.2.2 Total starch content

Total starch (TS) content was determined according to the method described by Englyst *et al*. (1996). A total starch assay kit (Megazyme International, Ireland) was used to determine the starch content. Samples (100 mg) were pre-dissolved in 2 M KOH at 4°C and pH was then adjusted with acetate buffer. Starch was hydrolysed (thermostable α-amylase, amyloglucosidase) in a water bath at 50°C. Liberated glucose was analysed using the glucose oxidase–peroxidase assay kit (K-GLUC, Megazyme, Ireland), and TS was calculated as glucose × 0.9. The total starch content was expressed as gram per 100 g dry weight (DW) of the sample.

2.3 Statistical analysis

A factorial design was used during a preliminary study which involves a few numbers of times for pre-treatment (data was not shown). However, this manuscript was only discussed to compare different treatments and times. Hence, the statistical analysis involves Analysis of Variance (ANOVA). The obtained data were analysed using Minitab 16 to determine the level of significance ($p<0.05$).

3. Results and discussion

The starch digestibility of *Kuini*'s kernel powder is shown in Table 2. After heat treatment, starch digestibility was significantly decreased especially for S10, S15 and B10, as compared to CP. After 180 mins of digestion, CP achieved an almost 39% rate of digestion compared to the heat-treated sample (ranging between 8 to 28%). Thermal treatments cause important modifications in the starch granules. For untreated samples, this fact is related to a non-gelatinized starch structure which is less resistant to enzymatic digestion (Urooj *et al*., 1999). Thus, more starch was digested compared to other samples. The steaming and blanching process causes changes in starch crystalline structure and makes it more susceptible to enzymatic hydrolysis. But upon cooling, starch undergoes a retrogradation process, where all starch becomes more crystalline and increases its resistance to the digestive enzyme (Narwojsz *et al.*, 2020). This explained why the starch digestibility of heat -treated *Kuini*'s kernel powder is lower than the un-treated sample. The hydrolysis curve of all *Kuini*'s kernel powder is shown in Figure 2. Heat-treated samples (B10, S10 and S15) showed the lowest rate of starch digestibility during a period of 30 to 180 mins compared to other samples. This result showed that most of the heat-treated samples were less digested compared

Table 2. Starch digestibility of *Kuini*'s kernel powder

Time (min)	Total starch hydrolysis (%)				
	Kuini's kernel powder (CP)	*Kuini*'s kernel powder (S5)	*Kuini*'s kernel powder (S10)	*Kuini*'s kernel powder (S15)	*Kuini*'s kernel powder (B10)
0	0.00	0.00	0.00	0.00	0.00
30	17.56^{a}	12.32^{a}	4.14^{b}	4.28^{b}	5.19^{b}
60	27.38^{a}	19.63^{a}	5.45^{b}	6.57^{b}	7.02^{b}
90	32.86^{a}	23.97^{b}	5.86^{d}	7.80^{c}	7.66^{cd}
120	35.93^{a}	26.54^{a}	5.99^{b}	8.46^{b}	7.89^{b}
150	37.64^{a}	28.07^{a}	6.03^{b}	8.81^{b}	7.97^{b}
180	38.60^{a}	28.97^{a}	6.04^{b}	9.00^{b}	8.00^{b}

Values with different superscripts within the same row are significantly different ($p<0.05$) (n = 3)

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to the untreated sample.

Results for the estimated glycaemic index of *Kuini*'s kernel powder are shown in Table 3 below. CP can be classified as a medium glycaemic index food (eGI 60), based on its eGI value. Sandhu *et al*. (2008) reported that mango kernel starch has a low GI value, 48.8 and 50.9 for two different varieties, Chausa and Kuppi. Meanwhile, heat-treated (steamed or blanched) *Kuini*'s kernel powder can be classified as low GI food as their eGIs were below 55. The low GI values of heat-treated *Kuini*'s kernel powder could be explained via incomplete gelatinization and retrogradation of starch, preventing the action of amylases in the starch, as previously described by Leoro *et al*. (2010). Low-GI and medium-GI foods are slowly digested, absorbed and metabolised resulting in stable fluctuation in blood glucose levels. On the other hand, high-GI foods are rapidly digested, absorbed and metabolised resulting in marked fluctuation in blood glucose levels.

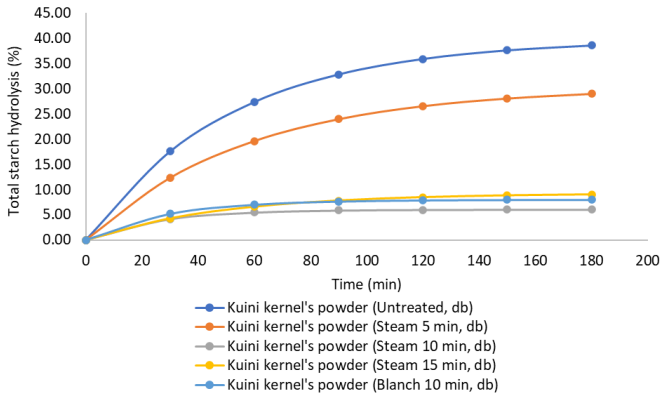

Figure 2. Enzymatic kinetics for in vitro glycaemic index of a *Kuini*'s kernel powder.

Zhang *et al*. (2018) studied the effect of physical modification on the starch digestibility, hydrolysis kinetics and estimated glycaemic index of jackfruit seed starch. They found out that the improved extrusion cooking process significantly increased the estimated glycaemic index of raw jackfruit seed starch, from 63.31 to a range of 91.20-98.78. The improved extrusion cooking technology has broken the starch granule and converted the resistant starch (RS) and slowly digestible starch (SDS) to a rapidly digestible starch (RDS) fraction. The direct heat treatment of the extrusion process has transformed jackfruit seed starch from medium GI food to high GI food.

Kuini's kernel contains starch between 15.93% to 28.40%. The starch content of *Kuini*'s kernel powder is relatively low compared to other seeds as reported by Zuwariah *et al*. (2018) for jackfruit seed (55.9%), Tananuwong *et al*. (2002) for jackfruit seed (77.76%), Fateatun Noor *et al*. (2014) for jackfruit seed (around 80%) and Manisha and Sikdar (2015) for mango seed (59.06%). No significant differences were observed in the total starch content for untreated and heat-treated samples, except for S5 (Table 3). Determination of total starch content is very important as the starch in food will affect the swelling, gelatinization behaviour, pasting properties, starch processing and food formulation (Tian *et al*., 1991).

Hydrolysis Index (HI) is a good tool for comparing the starch digestibility of different food samples affected by processing parameters. This index expresses the starch digestibility in any food against the starch digestibility in a reference material, namely glucose solution. Glucose solution is also used as the reference of 100% starch digestibility to calculate the GI. This is defined as the incremental postprandial blood glucose area after ingestion of the test product as a percentage of the corresponding area after ingesting an equicarbohydrate portion of the reference product (Jenkins *et al*., 1981). Similarly, the HI is also defined as the area under the starch hydrolysis curve of a test material expressed as a percentage of the corresponding hydrolysis area of the reference product (Goni *et al*., 1997). Goni also recommended using the latter to predict the glycaemic responses to foods due to the difficulty of assaying *in vivo* the GI of every foodstuff. Thus, from the measurement of the *in vitro* starch digestion rate and the calculation of the corresponding area under the hydrolysis curve (using Equation 1), the HI is calculated and used to estimate the corresponding predicted GI using Equation 2. As expected, the samples with the highest in vitro starch digestion rate would also elicit the highest glycaemic response as suggested by their eGI value (Table 3).

Table 3. Hydrolysis index (HI), estimated glycaemic index (eGI) and total starch content of Kuini's kernel powder

Sample (dry basis)	Hydrolysis index	eGI value	Total starch content
Un-treated	37.1538	60	19.6±1.38^{bc}
Steam 5 min	27.4978	55	28.40±1.86^{a}
Steam 10 min	6.8759	43	15.93±0.59^{c}
Steam 15 min	10.1274	45	16.79±0.57^{bc}
Blanch 10 min	7.6021	44	20.18±0.77^{b}

Values with different superscripts within the same column are significantly different ($p<0.05$) (n = 3)

These findings suggested that heat treatment decreases the digestion rate. Therefore, it slows the release of glucose into the bloodstream and reduces the HI. The *Kuini*'s kernel powder obtained as a by-product from *Kuini*'s processing industry can be considered as medium and low glycaemic index food, according to the classification defined by Brand-Miller *et al*. (1996).

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4. Conclusion

From this study, thermal processing significantly affected starch digestibility and estimated GI of *Kuini*'s kernel powder. Different heat treatments such as steaming and blanching had an impact on the *in vitro* starch digestibility. Heat-treated *Kuini*'s kernel powder could be promoted as a low GI food ingredient. It could be incorporated into food products such as bakery products (cake, cookies and biscuits), noodles and pasta. It is suggested to steam the kernel for at least 5 mins or to blanch for at least 10 mins to achieve low GI *Kuini*'s kernel powder.

Conflict of interest

The authors declare no conflict of interest.

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