Comparison of boba pearls made from tapioca starch and other unconventional flours and starches: Their glycaemic response (GR)

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ABSTRACT

Introduction: Boba milk tea, also recognised as bubble tea, is a popular beverage in Asia. The primary component in bubble tea is "boba" or "pearl" balls, made of tapioca starch. However, much remains to be seen if tapioca boba pearls have a profound impact on blood glucose. Methods: In a randomised, controlled crossover, single-blinded design study, 12 healthy Chinese male adults (body mass index 21±14 kgm⁻²) attended four sessions. At each session, bubble tea consisting of boba pearls made from tapioca starch (TS), sago starch (SS), high-amylose starch + sago starch (HA), or kithul flour + sago starch (KF) were served. Boba milk tea was served at breakfast, with volunteers consuming them in a fasted state at each session. The postprandial glycaemic response and insulin response were compared within participants. Results: There were observed differences at time 180min for incremental glucose between HA and SS (p=0.005), and for TS and SS for incremental insulin (p=0.004). Glucose iAUC was lower for TS compared to the other boba pearl treatments, although not significantly (p=0.093). There was no significant difference in iAUC of insulin (p=0.104) between the four boba pearl milk teas. **Conclusion:** With limited scientific research conducted on bubble milk tea, our study was the first to document the glycaemic responses of tapioca starch boba pearls and boba pearls made using unconventional flours and starches. The findings from this study is an important first step for future work to develop healthier boba pearls for bubble tea.

Keywords: boba pearls, flours, glycaemic response, insulinaemic response, starches

INTRODUCTION

Boba milk tea, otherwise recognised as bubble tea, has gained immense popularity globally, especially in Asia. The primary component in this beverage is "boba pearl". Boba pearl is commonly made from tapioca starch and has a chewy texture after cooking. It is usually soaked in a sugar syrup, after which is added to a sucrose-sweetened milk tea just before being served. The health implications of consuming boba milk tea, on its own, has been reported in a few studies, with a focus on the implications

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for obesity risk due the sugars present in the beverage (Min, Green & Kim, 2017; Pei *et al.*, 2018). Hence, boba milk tea is sold with varied proportions of sugar, so that consumers are given an option to reduce sugar level or to have it with no sugar.

Tapioca boba pearls not only increase the calories in bubble tea, but tapioca starch (TS) is putatively high in glycaemic index (GI) (Ramdath *et al.*, 2004; Remya, Jyothi & Sreekumar, 2018). It is known that high GI foods elicit a relatively large postprandial rise in glucose and insulin levels (Brand-Miller *et al.*, 2009; Ludwig, 2002). With the consumer shift towards healthier foods and a concomitant increase in the consumption of boba milk tea, it is crucial to produce alternatives for tapioca pearls. This will enable manufacturers to improve the overall nutritional properties of boba milk tea.

Tapioca pearl (made from tapioca starch) is derived from the roots of a cassava plant (*Manihot esculenta*) (Tonukari, 2004). Starch is an essential food ingredient in many food products, especially in Asia, where many food products are highly carbohydrate-based. For example, sago starch (SS) is used in the production of biscuits, noodles and bread (Karim *et al.*, 2008), and more than two-thirds of cassava (tapioca) starch production is used for human consumption (Tonukari, 2004).

Therefore, there is a need to produce starchy foods that are low in GI, i.e. slowly digested, thereby leading to a small and gradual rise in blood glucose. In a previous study, Ng & Henry physiochemical (2020)studied the characteristics unconventional of starches used in Asia and it was found that high amylose maize was highly stable, requiring higher gelatinisation temperature, while kithul flour (KF) (Caryota urens) had a digestion rate that was significantly lower than other conventional starches/flours.

The postprandial glycaemic responses (GR) of consuming bubble tea have not been tested; this study therefore will pave the way to formulate 'healthier' and palatable forms of boba pearls for consumers. In selecting our alternative sources of carbohydrate, it was important to recognise and be cognisant of the unique texture and mouthfeel of the tapioca-based boba pearls. Therefore, the alternative carbohydrate sources investigated were: KF (Caryota urens), SS (Metroxylon saqu), and high amylose maize starch (HA). These carbohydrates were chosen on the basis of their wide availability and application in producing a variety of Asian-based snack foods.

Taking into consideration the above factors, this study, for the first time, aims to compare the GR of conventional tapioca boba pearls and other boba pearls formulated using unconventional flours and starches. These different boba pearls will be formulated in a standardised milk tea concoction and consumed with a snack, as a holistic eating event.

MATERIALS AND METHODS

Boba pearl flours and starches

TS (Ng Nam Bee Marketing, Singapore), SS (Yiak Say Hang Food Industries, Singapore), HA (HI-MAIZE[®] 260, Ingredion, Singapore), and KF (Kandy, Sri Lanka) were used in the making of boba pearls.

Boba pearl making

Preliminary work was done using a systematic method for the formulation of boba pearls. Boba pearls were made by mixing the starch or flour with boiling water according to the formulation stated in Table 1. The mixture was kneaded to form a dough which is rolled and shaped into a long and thin log (diameter: 1cm). A dough cutter was used to cut the log into small pieces (length: 5mm) and each piece was rounded into a small ball with an average weight of 0.75±0.05g.

TS and SS were used without any blending as they were able to form a dough-like structure when mixed with boiling water. For the blended variants, a proportion of SS was mixed with KF or HA starch until a perfect dough-like structure was formed when boiling water was added. Cooking time was also predetermined from these trials to ensure that the starches were cooked thoroughly to an optimal level of chewiness. The amount of cooking time, flour(s) and water used for each treatment were recorded and shown in Table 1.

Total starch and amylose content

The total starch content of boba pearls was measured by an enzymatic technique using the Megazyme assay kit (K-TSTA, Megazyme International, Ireland). Samples were first incubated with thermostable α -amylase at 100°C to hydrolyse starch into maltodextrins. For the high amylose maize variant, cold 1.7M sodium hydroxide was used to pre-dissolve the resistant starch present, and sodium acetate buffer was used to neutralise the sample before the addition of thermostable α -amylase. Amyloglucosidase was then added to hydrolyse maltodextrins into D-glucose. For quantitative measurement, glucose oxidase/peroxidase (GOPOD) reagent was then added and the absorbance of the samples was determined using а UV spectrophotometer (UV-2600, Shimadzu, Japan).

The amylose of content the boba pearls was measured using the amylose/amylopectin assav kit Megazyme (K-AMYL, International, Ireland). Samples were first dispersed in dimethyl sulphoxide (DMSO) at 100°C and then ethanol was added to remove the lipids. The precipitated sample was dispersed in DMSO before dissolution in an acetate and salt buffer. An aliquot of

Table 1. Formulation of boba pearls	and milk	tea						
Boba Pearls	Starch/ Flour 1	Amount (g)	Starch/ Flour 2	Amount (g)	Water (g)	Cooking time (mins)	Cooked weight of pearls (g)	Total available CHO (g)
A: Tapioca Starch (TS)	TS	54.0		,	32.4	7	86.4	50
B: Sago Starch (SS)	SS	57.4	ı	ı	33.3	7	90.8	50
C: Kithul flour (KF) [†]	KF	41.5	SS	17.8	33.6	7	92.9	50
D: High amylose maize starch (HA) [†]	HA	38.1	SS	38.1	57.2	13	133.4	50
Milk Tea & Bread		Amount (g)	Total amount (ml)	Total available CHO (g)				
Tea		246.8	300	0				
Evaporated milk		37.0		4.6				
Sugar syrup		16.0		10.7				
White bread		47.8	47.8	25				
*Blends with sago starch (SS)								

the dissolved sample was taken for the measurement of total starch later. To another aliquot of the dissolved sample, lectin concanavalin A (Con A) was added to complex the amylopectin which was removed by centrifugation. An aliquot of the supernatant was taken for the measurement of amylose. An enzyme mixture of amyloglucosidase and α -amylase was added to both the amylose and total starch aliquots for hydrolysis into D-glucose. GOPOD reagent was then added and the absorbance of the samples was measured. The concentration of amylose was estimated as the ratio of absorbance of the supernatant aliquot to that of the total starch aliquot.

Total available carbohydrate (TAC) content

The total available carbohydrate (TAC) content of boba pearls was measured enzymatic technique bv an using the Megazyme assay kit (K-ACHDF, Megazyme International, Ireland). The flours were first incubated at 80°C with α -amylase to gelatinise, hydrolyse, and depolymerise non-resistant starch. They were further incubated at 60°C with protease to solubilise and depolymerise proteins. Amyloglucosidase was also added to hydrolyse starch fragments into D-glucose. Following enzymatic hydrolysis. the absorbance of the samples was measured using a UV spectrophotometer (UV-2600, Shimadzu, Japan) to determine D-glucose and D-fructose. The TAC (%) present in the flour is derived from the sum of D-glucose content (%) and D-fructose content (%).

Texture analysis of boba pearls

The texture analysis of cooked boba pearls was analysed by performing two successive compressions using a texture analyser, TA-XTplus (Stable Micro Systems Ltd, Surrey, UK). A single boba pearl sample was compressed to a strain of 75% using a cylindrical probe $(\emptyset = 75 \text{ mm})$ with a trigger force of 5.0 g. The pre-test speed was 1 mm/second (sec) and both the test and post-test speeds were 5 mm/sec. The recovery time was 5 sec between the first and second compression. The analysis was carried out at room temperature. There were ten replicates for each sample. The subsequent parameters were achieved from the force-distance curves (Bourne, 1978): the peak force (N) of the first compression was determined as "hardness": the ratio of the area of the second and first compression was determined as "cohesiveness"; the ratio of the pearl's detected height in the second compression cycle to that of the first compression was determined as "springiness"; and the multiplication of the hardness, cohesiveness, and springiness values was identified as the "chewiness".

In vivo study

The research took place at the Clinical Nutrition Research Centre (CNRC) within the Singapore Institute for Food and Biotechnology Innovation (SIFBI), Agency of Science, Technology and Research (A*STAR), Singapore. The study was approved by the Domain Specific Review Board of the National Healthcare Group (2018/01194),registered under the Clinicaltrial.gov registry as NCT04115657. All procedures were conducted based on the guidelines stated in the Declaration of Helsinki.

Study population

Volunteers were recruited from the public. Anthropometric measurements (height, weight, waist-hip circumference, triceps and biceps skinfolds), blood pressure. and finger-prick fasting blood glucose measurements were collected. Females were not included to minimise disparities in the data because hormonal changes during of their menstrual cycle. Young, healthy Asian

Chinese males between the ages of 21 -40 years, with a body mass index (BMI) between 18.5 to 25 kg/m² and normal blood pressure (<140/90 mmHg) were included in the study. Those who were athletes/sportsmen, dieters, smokers, with a fasting blood glucose of >6 mmol/L, glucose-6-phosphate dehvdrogenase deficiency (G6PD deficiency), metabolic diseases, such as diabetes, hypertension etc., medical conditions and/or taking medications known to affect glycaemia (glucocorticoids, thyroid hormones, thiazide diuretics). intolerances or allergies to foods, were excluded from the study.

Sample size

Studies of the analysis of GR and GI in humans have been based on ten subjects, as reviewed by the Food and Agriculture Organization/World Health Organization (FAO/WHO, 1998) to take into account the inter-individual variations. A sample size of 12 was therefore considered adequate for the current study to account for interindividual variabilities.

Study design and experimental protocol

The study was a randomised, controlled, single-blinded cross-over design. Participants attended one screening visit and four test sessions (consisting of a milk tea with different types of boba pearls and a snack, which was white bread). For a controlled condition, the tapioca boba milk tea was used. The other three test boba milk teas were tested with similar formulations, except for the types of boba pearls used. All four test sessions were separated by a threeday washout period to minimise any cross-over effects. Randomisation of the sequence of treatments was determined through an online computer software (Randomizer.org).

Volunteers who were interested in the study and fulfilled all the inclusion criteria underwent a screening session. They were requested to come to the centre in the morning (fasted for at least ten hours). Informed consent was signed before basic anthropometric measurements were measured. Height was taken using a stadiometer (Seca Limited, Birmingham, West Midlands, Middlesex, body weight UK), and composition were obtained using the bioelectrical impedance analysis (BIA) machine (Tanita BC-418, Tokyo, Japan), and the Omron blood pressure monitor (Model Hem-907) was used to measure participants' blood pressure. The HemoCue 201+ Glucose RT analyser (HemoCue Ltd., Dronfield, UK) was used to measure finger-prick fasting blood glucose levels. Three days before the test session, volunteers were requested not to take part in rigorous activities and they were also told to avoid caffeine and alcohol the day before the test.

On test days, volunteers had to arrive between 8:30 am to 9:00 am after a 10to 12-hour overnight fast. They rested for ten minutes (min) before an indwelling intravenous cannula was inserted into a forearm vein, by a phlebotomy-trained state registered nurse. Throughout the test session, the line was kept patent with 3 mL non-heparinised saline. A 3 mL baseline venous blood sample (0 min) was obtained immediately after the insertion of the catheter. Subsequently, the volunteers were asked to consume the boba pearl drink and white bread within 15 min. After the test meal, 3 mL venous blood samples were collected at 15, 30, 60, 90, 120, 150, 180 min to measure plasma glucose and insulin concentrations. Volunteers were encouraged to remain desk-bound throughout the three-hour study period to minimise physical movement. After 180 min, the catheter was removed and the study session was completed. The same steps were repeated for all test visits.

Treatment meals

Volunteers were required to consume a standardised dinner the night before the test session. The standardised dinner consisted of a frozen, ready-toeat meal (Butter chicken with cumin rice, Chef-in-Box, Singapore) and a Milo tetra pack drink (Milo Chocolate Malt, Nestlé, Switzerland). Given that boba tea is a complex mixture of boba pearls, milk tea (that contains sucrose), and is usually consumed with a snack (a slice of bread), we decided to simulate the real-life situation in our experimental design. For each test day, participants consumed boba pearl milk tea along with white bread as a meal. Therefore, the amount of available carbohydrates provided by boba pearls was 50g, milk tea 10.7g, and bread 25g (Table 1).

All boba pearl milk teas were prepared in the CNRC food product development kitchen. Before each test day, boba pearls were prepared and then stored in a 4°C chiller. On the test day, these chilled boba pearls were then boiled in water according to a pre-determined cooking duration. After cooking, the pearls were strained into the serving cup, allowed to cool for a minute in an ice bath, after which chilled milk tea was added and the beverage was served immediately.

For the tea base of the drink, 12g tea leaves (Brooke Bond 3 roses, Unilever, UK) was boiled in 1.1L of water for one min and then simmered for another three min. Then, 150g of evaporated full cream milk (Carnation, F&N Holdings, Malaysia) was added into 1L of brewed tea together with 65g of 200% w/w sugar solution (SIS, Singapore). The milk tea was also made the day before and stored in a 4°C chiller. The formulation for one serving (300 g) of milk tea was 247g of brewed tea, 16g of sugar solution, and 37g of evaporated milk.

Blood analysis

Venous blood samples were collected at fixed time points in Vacutainers® (Belton Dickinson Diagnostics, NJ, USA) with disodium EDTA. These samples were then centrifuged at 1500 g for 10 min at 4°C (Sorvall[™] ST 16 Centrifuge, Thermo Fisher Scientific, Waltham, MA, USA), where plasma was obtained. Plasma was aliquoted into Eppendorf tubes and stored at -80°C until analysis. Plasma glucose was measured using the immunochemistry analyser COBAS (Roche, HITACHI, Los Gatos, c311 CA, USA), while plasma insulin was measured using the immunochemistry analyser COBAS e411 (Roche, HITACHI, Los Gatos, CA, USA). Inter- and intraassay CVs for glucose (<2% and <1.5%, respectively) and insulin (<6% and <5%, respectively) were determined by the manufacturers. Postprandial blood glucose concentration changes were measured by computing the difference between the fasting and the blood glucose concentration at a specific time interval. The trapezoidal rule was used to calculate postprandial glucose and insulin incremental area under the curve (iAUC), ignoring the area under the baseline (Wolever, 2006).

Data and statistical analysis

All data and figures were processed in a Microsoft Excel spreadsheet (Microsoft Corporation), presented as mean±*SEM* (standard error of the mean), unless otherwise stated. Data were tested for normality using the Sharpiro-Wilk test, as well as visually using Q-Q plot. Linear mixed effects procedure with treatment as fixed factor and subject as random effect was conducted to investigate the effect of treatment on the iAUC for glucose and insulin. The same procedure was used

Boba pearl sample	Total starch (% w/w d.w.b)	Amylose (% w/w d.w.b)	TAC (%)
TS	86.4±1.6	23.0±2.1	92.6±0.1
SS	85.2±0.5	24.3±0.4	87.0±0.9
KF+SS	88.7±0.6	20.6±2.2	83.1±1.1
HA+SS	90.1±4.5	40.5±2.0	53.3±1.1

Table 2 Total starch, amylose and total available carbohydrate contents of bobapearl samples

TS, tapioca starch; SS, sago starch; KF, kithul flour; HA, high amylose maize starch

to test for significant difference in mean fasting glucose and insulin values prior to the four treatments, as well as at each time point of the incremental glucose and insulin responses. Statistical significance was attained when p<0.05. All statistical analysis was done using IBM SPSS for Windows version 24.0 (IBM Corp, Armonk, NY, USA).

RESULTS

Chemical composition of raw materials

The chemical composition of all starches and flours used in the formulation of the boba pearls is derived from our previous work (Ng & Henry, 2020). All the starches contained trace amounts of proteins, except for HA. There was trace amounts of fat for SS and KF boba pearls. A high dietary fibre content was observed in KF and HA boba pearls.

Total starch, amylose content, and TAC content of boba pearls

Total starch, amylose content, and TAC content of the boba pearl samples are shown in Table 2. The amylose content

in HA boba pearls was almost twice the amylose content of the other boba pearl variants. The TAC analysis was conducted to determine the available carbohydrate content, so as to calculate the available carbohydrate of treatment meals for *in vivo* GR testing.

Texture analysis of boba pearls

The hardness, cohesiveness, springiness, and chewiness for the four types of cooked boba pearls were determined (Table 3). HA had much higher hardness, lower cohesiveness, and lower springiness as compared to the TS boba pearls. SS had similar textural parameters as TS boba pearls. KF boba pearls had higher hardness and chewiness compared to TS and SS pearls.

In vivo study

Baseline characteristics

For the present study, 12 young, healthy Chinese male adults fulfilled the study inclusion criteria and completed all four arms of the study. Their baseline characteristics are shown in Table 4.

Boba pearl sample	Hardness (N)	Cohesiveness	Springiness	Chewiness
TS	10.32±1.05	0.86±0.02	0.94±0.05	8.41±1.09
SS	11.62±1.54	0.86±0.01	0.95±0.04	9.44±1.30
KF	16.65±2.57	0.84±0.01	0.96±0.04	13.49±2.36
HA	28.57±2.51	0.47±0.01	0.72±0.05	9.80±1.51

Table 3 Texture parameters measured for boba pearls

TS, tapioca starch; SS, sago starch; KF, kithul flour; HA, high amylose maize starch

Anthropometric and physiological parameters	Mean±SD
Age (years)	24.8±1.8
Height (cm)	173.9±6.4
Weight (kg)	64.7±7.4
BMI (kg/m ²)	21.3±14.8
Systolic blood pressure (mmHg)	122.7±8.0
Diastolic blood pressure (mmHg)	73.2±6.6
Waist circumference (cm)	74.1±5.1
Hip circumference (cm)	94.5±4.8
Fasting blood glucose (mmol/L)	4.5±0.4

Table 4 Characteristics of study participants (n=12). Data presented as mean±SD (standard deviation)

BMI, body mass index

Glucose and insulin responses

Postprandial glucose responses of the four treatments are shown in Figure 1A and the postprandial insulin responses to the four treatments are shown in Figure 1B. All treatments produced an early rise in plasma glucose concentration with TS and HA having the highest peak level around 30 min, while SS and KF showed a later peak for blood glucose, i.e. 45 min after consumption followed by a gradual decline (Figure 1). At the 180min time point, there was a significant difference between HA and SS for incremental glucose (p=0.003). When presented as iAUC, TS appeared to have the lowest reduction in glucose response, but overall no significant difference was found between all treatments (p=0.093). There were significant differences observed in incremental insulin at time points 90 (p=0.031) and 180min (p=0.004). At 180min, incremental insulin values of SS was significantly lower than that of TS (*p*=0.016) and HA (*p*=0.006). However, after Bonferroni correction, there was no significant difference between the treatments at time point 90min. In addition, the iAUC for insulin (p=0.104)did not show significant differences between the four boba pearl milk teas.

DISCUSSION

There is a growing demand for starchy foods to be more slowly digested and metabolised, leading to a more gradual and smaller rise in blood glucose. Hence, the purpose of this study was to investigate whether tapioca starch boba pearls may elicit a high GR and whether boba pearls developed from unconventional starches may reduce the GR compared to tapioca boba pearls. Given boba tea's wide popularity, and the potential impact of consuming high GR foods on metabolic health, our study was a first attempt to examine the postprandial glycaemia of boba milk tea.

Before commencing *in vivo* work, alternative starches and flours for new boba pearls were formulated for comparison with tapioca boba pearls. Our previous work showed KF and HA starches to have significantly lower rates of digestibility as compared to the other starches, explained by the higher amounts of protein, amylose, and dietary fibre present in them (Grace *et al.*, 2020). Hence, these flours and starches were selected to create new boba pearls. The formulation of boba pearls was conducted in a systematic way to ensure that a perfect dough-like



Figure 1 represents the incremental glucose (A) and insulin (B) curves for 180 min. The bar plots on the right-hand side represent values as mean±SEM; n=12. iAUC180 was calculated using the trapezoid rule ignoring the area below the baseline. Total iAUC180 corresponds to the area under the curve for the entire 180 minutes of measurement. p>0.05* indicates time point at which there was significant difference between the incremental values. TS, tapioca starch; HA, high amylose maize starch; KF, kithul flour; SS, sago starch

structure was formed. This was a crucial step in ensuring that the boba pearls formed using alternative sources of carbohydrate were cognisant with the textural qualities of tapioca starch boba pearls. TS and SS were able to form without any blending. On the other hand, HA and KF were blended with SS. The lower starch content, and the higher fat, protein, and dietary fibre contents in these flours, as well as the higher amylose to amylopectin ratio in HA resulted in higher pasting temperatures and decreased gelatinisation (Grace et al., 2020, Tian et al., 2019). This contributed to the inability for HA and KF starches to form a dough-like structure when mixed with boiling water. Therefore, to form boba pearls using KF and HA, SS was required in the blend to act as a binder in order for these variants to be able to form a dough-like boba pearl perfectly.

Textural parameter findings showed that HA boba pearls had much higher hardness, lower cohesiveness, and lower springiness as compared to TS boba pearls. This may be attributed to the incomplete gelatinisation and restricted swelling of the starch granules due to limited water absorption in the HA boba pearls (Cornejo-Ramírez et al., 2018). Compared to TS and SS, KF had a greater amount of protein and dietary fibre, which contributed to its higher hardness. Although there were some differences in the texture of the boba pearls, the instrumental measurement could be more sensitive in distinguishing textural properties than the sensory perception in humans (Truong et al., 2002).

The iAUC glycaemic response results although not statistically significant, saw an overall greater attenuation of glucose response with TS and SS compared to TS and HA. Time point 180 min saw significant differences in glucose response between HA and SS (p=0.003).

This could be due to the large amount of dietary fibre (resistant starch) and high amylose content present in HA that may have a large influence on attenuating the glucose response (Ingredion, 2020). SS and KF have lower amylose content, with SS containing between 24 to 31% amylose content (Ahmad et al., 1999). This could be a possible explanation for the higher glucose iAUC observed in SS and KF compared to HA and TS boba pearls. This indicates that the amylose content of starches and flours used in formulating boba pearls may play an important role in attenuating glucose response. The molecular structure of amylose is tighter and more compact, thus less susceptible to breakdown than amylopectin whose structure is more vulnerable to digestion. Therefore, the amylose content of starches and flours may influence both the textural qualities and glucose attenuating properties of boba pearls. This may be an important consideration when developing and formulating boba pearls.

A possible explanation the for treatments showing no statistical significance could also be due to the combination of consuming other nonstarch ingredients together that may have an interaction with boba pearls. Non-starch ingredients, such as tea, which is rich in polyphenols, such as catechins and tannins, may decrease starch digestibility and possibly blood glucose response by inhibiting enzymes and interacting with starch (Thompson & Yoon, 1984). Future studies should consider using a liquid-base component that is not rich in polyphenols, and/ or other ingredients known to affect glycaemia.

Differences in the habitual mastication of starchy foods can also contribute to glycaemic variations (Ranawana *et al.*, 2011, Ranawana *et al.*, 2010). Volunteers were asked to

consume boba milk tea with a large straw, through which the boba pearls were slurped and chewed. However, the number of chews per boba pearl was not controlled for during this study. Hence, this could be a confounder in the study as factoring the number of chews each volunteer takes to masticate each pearl is different and this could have affected the rate of starch digestion. It is also unclear if the small textural differences between the boba pearls may have an effect on the masticatory sequence, which may have influenced the GR of the chewed particles (Bornhorst & Singh, 2012, Miwa, Shiga & Kobavashi, 2001). Thus, in addition to replacing the liquid-base component of the beverage, future studies could also focus on the mastication process of boba pearls in determining the glycaemic response of a boba drink. Finally, given that there is some contribution of lactose from evaporated milk in bubble tea, a criteria to test participants for lactose intolerance should be included. A small number of Asian Chinese might have the ability to break down lactose and this may contribute to postprandial glucose response. These factors and limitations may have led to differences in glycaemic and insulinaemic responses, and should therefore be considered for future work in this area.

CONCLUSION

Building on a real-life situation where boba tea is a complex mixture of pearls and milk tea, consumed with a snack, our study was the first of its kind to examine the effects of various boba pearls when consumed as a holistic eating event. Using tapioca starch and a range of unconventional flours and starches in boba pearl manufacture, our study showed that there was no statistical significance, but the results

were nonetheless still meaningful and warrants further investigation. An understanding of the impact on GI would make it more useful to gauge its practical relevance. Our findings enhanced the research in this field and is a necessary first step in understanding how this popular beverage fits in the landscape of sugar-sweetened beverages in the Asian region. With limited published data on the GR of boba pearls consumed in this region, this study is timely as it sets a firm foundation upon which future work can be based on.

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Authors' contributions

BK, implemented and performed the experiments and human studies, analysed and interpreted the data, responsible for the statistical analysis and interpretation of data, and wrote the manuscript; RYCQ, implemented and performed the experiments and human studies, analysed and interpreted the data, responsible for the statistical analysis and interpretation of data, and wrote the manuscript; GCFN, responsible for the conception and design of the study, implemented and performed the experiments and human studies, analysed and interpreted the data, and wrote the manuscript; SP, analysed and interpreted the data, responsible for the statistical analysis and interpretation of data, and wrote the manuscript; CJH, responsible for the conception and design of the study, and wrote the manuscript. All authors read and approved the manuscript and had full access to the study data and shared the final responsibility for the decision to submit this report for publication.

Conflicts of interest

All authors declare no conflict of interest.

References

- Ahmad FB, Williams PA, Doublier JL, Durand S & Buleon A (1999). Physico-chemical characterisation of sago starch. Carbohydr Polym 38:361-370.
- Bornhorst GM & Singh RP (2012). Bolus Formation and Disintegration during Digestion of Food Carbohydrates. *Compr Rev Food Sci Food Saf* 11:101-118.
- Bourne MC (1978). Texture profile analysis. *Food Technol* 32: 62-66.
- Brand-Miller J, Mcmillan-Price J, Steinbeck K & Caterson I (2009). Dietary glycemic index: health implications. J Am Coll Nutr 28:446S-449S.
- Cornejo-Ramírez YI, Martínez-Cruz O, Del Toro-Sánchez CL, Wong-Corral FJ, Borboa-Flores J & Cinco-Moroyoqui FJ (2018). The structural characteristics of starches and their functional properties. *CYTA J Food* 16:1003-1017.
- FAO/WHO (1998). Carbohydrates in human nutrition: report of a joint FAO/WHO expert consultation. FAO Food Nutr Pap 66:1-140.
- Ingredion (2020). In: Everyday nutrition with HI-MAIZE® 260 resistant starch. From https:// apac.ingredion.com/ingredients/emea/ himaize-260-22000b01.html [Retrieved 4th March 2020].
- Karim A, Nadiha M, Chen F, Phuah Y, Chui Y & Fazilah A (2008). Pasting and retrogradation properties of alkali-treated sago (Metroxylon sagu) starch. Food Hydrocoll 22:1044-1053.
- Ludwig DS (2002). The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA* 287:2414-2423.
- Min JE, Green DB & Kim L (2017). Calories and sugars in boba milk tea: implications for obesity risk in Asian Pacific Islanders. *Food Sci Nutr* 5:38-45.
- Miwa M, Shiga H & Kobayashi Y (2001). The effect of food hardness on chewing movement. *J Jpn Soc Mast Sci Health Prom*:85-93.
- Ng GCF & Henry CJ (2020). The Physicochemical Characterization of Unconventional Starches and Flours Used in Asia. *Foods* 9:182.

- Pei YL, Chen TC, Lin FY, Doong JY, Chen WL, Kamoshita S, Sari IK, Takeichi H & Yamamoto S (2018). The Effect of Limiting Tapioca Milk Tea on Added Sugar Consumption In Taiwanese Young Male and Female Subjects. *J Med Invest* 65:43-49.
- Ramdath DD, Isaacs RL, Teelucksingh S & Wolever TM (2004). Glycaemic index of selected staples commonly eaten in the Caribbean and the effects of boiling v. crushing. *Br J Nutr* 91:971-977.
- Ranawana V, Clegg ME, Shafat A & Henry CJ (2011). Postmastication digestion factors influence glycemic variability in humans. *Nutr Res* 31:452-459.
- Ranawana V, Monro JA, Mishra S & Henry CJK (2010). Degree of particle size breakdown during mastication may be a possible cause of interindividual glycemic variability. *Nutr Res* 30:246-254.
- Randomizer.Org. From http://www.randomizer. org/ [Retrieved February 22 2019]
- Remya R, Jyothi AN & Sreekumar J (2018). Effect of chemical modification with citric acid on the physicochemical properties and resistant starch formation in different starches. *Carbohydr Polym* 202:29-38.
- Thompson LU & Yoon JH (1984). Starch digestibility as affected by polyphenols and phytic acid. *J Food Sci* 49:1228-1229.
- Tian J, Ogawa Y, Shi J, Chen S, Zhang H, Liu D & Ye X (2019). The microstructure of starchy food modulates its digestibility. *Crit Rev Food Sci Nutr* 59:3117-3128.
- Tonukari NJ (2004). Cassava and the future of starch. *Electron J Biotechnol* 7:5-8.
- Truong VD, Daubert CR, Drake MA & Baxter SR (2002). Vane Rheometry for Textural Characterization of Cheddar Cheeses: Correlation with Other Instrumental and Sensory Measurements. LWT- Food Sci Technol 35:305-314.
- Wolever TM (2006). The glycaemic index: A physiological classification of dietary carbohydrate, Cabi.