Original Article

Evaluation of the First Radiolabeled ^{99m}Tc-Jerusalem Artichoke-Containing Snack Bar on Gastric Emptying and Satiety in Healthy Female Volunteers

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Background: Jerusalem artichoke [JA] is a rich source of dietary fiber. Previous reports showed that JA influence delayed gastric emptying by prolonging gastric emptying, resulting in extending satiety.This property may be beneficial for current lifestyle of the working women who need simple and convenient form of energy supply but provide the effect of weight controlling at the same time.

Objective: The present study aimed to label JA with ^{99m}Tc-pertechnetate and to evaluatethe effect of JA formulation in a snack bar (JASB) on gastric emptying and satiety in healthy female subjects.

Materials and Methods: JASB and control snack bar [CSB] were prepared by mixing JA syrup with $SnCl_2$ 0.1 mg for 15 minutes, and subsequently adding ^{99m}Tc-pertechnetate 1 mCi. The incubation time was 10 minutes at room temperature. The labeled ^{99m}Tc-JASB mixture were then put in a rectangular mold and baked in an oven at 180°C for 20 minutes. A randomized double-blinded crossover design study was conducted to evaluate the gastric emptying process in vivo. Thirty healthy female participants whose average age was 29.1$\pm$1.95 year were recruited for this study. The participants were instructed to consume JASB and then to stand in an upright position. For gastric emptying analysis, scintigraphic method was used. Sequential data acquisition was performed in upright 45 degree left anterior oblique (45° LAO) position for 4 hours. Participants were also asked to describe their satiety by making a vertical mark on 100-mm visual analogue scales [VAS] during data acquisition. This procedure was repeated for each subject but after consuming CSB.

Results: The ^{99m}Tc-Jerusalem artichoke was successfully labeled with radiochemical purity of more than 90%. The gastric emptying half time and percent gastric of JASB were significantly greater than CSB ($p<0.05$). The satiety at these intervals were also notably distinctive ($p<0.05$) between JASB and CSB. In JASB studies, the gastric emptying exhibited a strong positive correlation with satiety ($R^2 = 0.89$, $p<0.05$).

Conclusion: JA plays a key part in delaying gastric emptying of consumed snack bar, resulting in prolonged subjects' satiety.

Keywords: Jerusalem artichoke, Tc-99m, Gastric emptying, Satiety, Dietary fiber

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Trend in current everyday lifestyle in evitably encourages over consumption of highly caloric food that is not only rich in fat but also carbohydrate, and protein. Combined with lack of physical activity, this lifestyle adversely results in overall energy imbalance[1]. Inappropriate eating habit is also contributing to a wide range of health risks, especially overweight, obesity, chronic respiratory diseases, heart disease, stroke, cancer and diabetes[2,3]. Indeed, the prevalence of overweight and obesity in particular has rapidly escalating worldwide. Specifically in 2014, 266 million men and 375 million women were obese, compared to merely 34 and 71 million, respectively, in 1975. If this trend continues, global obesity incidence could reach 18% and surpass 21% of men and women populations by 2025[2].

To mitigate those risks, properly controlled dietary such as food with high nutrients but low in calories should be made available. Furthermore, prolonging satiety after consumption may help individual eating less, reducing overall energy intake and hence chances of inducing bad eating habits, related to health problems.

Satiety is a state where hunger and further eating are inhibited due to consuming food[4,5]. Assisting people to feel satiety is an effective means of decreasing inter-meal hunger and therefore reducing overall energy intake[4]. Satiety is known to be influenced by various factors, e.g., energy load, amount of nutrients; fat, carbohydrate, protein in food[6], and physiological processes in brain and body such as gastric emptying [GE][7]. GE is an evacuation of food from stomach to duodenum. It is one of determining mechanisms for satiety,that is,the slower the rate of GE, the longer feeling of satiety[8]. Dietary fiber [DF], the nutrient in the diet that is not digested in gastrointestinal tract, is listed on the Nutrition Facts panel on food products de jure. Food products that have 2.5 g DF are claimed as good source of fiber, while food products that have 5.0 g DF are claimed as excellent source of fiber[9]. DF is believed to slowdown GE, to suppress appetite, to increase satiety, and to decrease energy intake[10-12]. Higher intakes of DF are therefore often linked to lower body weights and less chance of acquiring cardiovascular diseases[9].

Inulin or inulin-type fructans is a polysaccharide whose chemical structure is a linear biopolymer of D-fructose units, connected by β (2,1) glycosidic linkages and terminated with one D-glucose molecule, and linked to the fructose chain by an α (2,1) bond[13,14]. Thanks to this β (2,1) linkages between the fructose monomers, inulin is resistant to human digestion. It is hence considered as an effective prebiotic or DF. Nonetheless, it can be fermented by health-promoting bacteria in colon such as bifidobacteria or lactobacilli, resulting in increasing faecal biomass and water content of the stools[15]. Inulin can be found in many vegetables and fruits. JA tuber, for example, is a rich source of inulin. Typical dried JA tuber contains inulin up to 50%[16]. JA has as such increasingly been used as functional food ingredients as an instigating agent of probiotic bacteria growth[17-19]. Thus far, its role in gastric emptying as a satiety prolonging agent has remained yet to be evaluated.

In addition, increasing level of sedentariness also plays a majorpart in encouraging the existing obesity issue. Women with full-time desk jobs (18 to 59 years old) are having high daily levels of sitting ($\geq$7.4 h/day) but much less physical activities[20,21]. Therefore, compared to adult men counter parts, they are about as half as likely to be overweight. Owing to their job demand, those working women often depend on highly nutritious and tasty snacks that can temporarily remedy their hunger until the next meal[22]. Rich DF snack bar, being able to resist digestion, perfectly fits these criteria and may thus serve as a key element in their weight control program. Motivated by this measure, the authors were therefore interested in developing Jerusalem Artichoke Snack Bar [JASB] that could delay gastric emptying process, while enhancing satiety in healthy and actively working women. Accordingly, this study aimed to assess the gastric emptying process after JASB consumption. To this end, quantitatively determining the relevant parameters calls for radiolabeling of gastric content being digested. The proposed evaluation of JASB properties by means of nuclear medicine imaging modality, to the best of our knowledge,was yet the first non-invasive study to report inulin labeling in JA in vivo.

Materials and Methods

Participants

Thirty healthy female participants were randomly recruited from those currently working at local universities, companies, and governmental offices. All of them must have met the inclusion criteria: age ranging between 18 to 59 years old, apparently healthy, not pregnant, no known neither metabolic diseases nor chronic gastrointestinal disorders and not in any prescribed nor weight losing diet. Those who were reported of being allergic to the snack bar ingredients, lactating or undergoing any medical treatment that may affect gastrointestinal tract were excluded. Prior to participating in the present study, all subjects had given written informed consent. The study was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Snack bar preparation and radiolabeling

JASB (40 g) consisted of dried JA tuber, dried fruit (banana, apple, strawberry), roasted pumpkin seed (Flower Food Ltd.,Part, Bangkok, Thailand), orange juice, whole grain oat flour (Bob's Red Mill Natural Foods, OR, US), JA flour, JA syrup (JA flour and JA syrup were provided by the Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Thailand), and Fiber Gum (Rama Production Co., Ltd., Bangkok, Thailand). Dried JA tuber and dried fruits (banana, apple and strawberry) were earlier prepared by dehydrating at 70°C for 7 hours. To radiolabel the inulin in JA for scintigraphy, dehydrated $SnCl_2$ 0.1 mg was added to JA syrup and mixed for 15 minutes. ^{99m}Tc-pertechnetate 1 mCi was subsequently added to the mixed JA syrup. Incubation time was 10 minutes at room temperature. Labeled ^{99m}Tc-JASB was used as coalescing material for both liquid and solid ingredients. The JASB mixture were then put in a rectangular mold and baked in the oven at 180°C for 20 minutes, after which it was cooled down to the room temperature. Each participant was given one of these JASBs to consume with 250 ml of water.

Control snack bar [CSB] were prepared by using the same formula as JASB except that dried JA tuber, JA flour, and JA syrup were instead replaced bycrispy rice (Kellogg's Rice Krispies® Cereal, Kellogg Co., US), whole wheat flour, and glucose syrup, respectively. Similarly, dehydrated $SnCl_2$ 0.1 mg was added to glucose syrupand mixed for 15 minutes, subsequently added with ^{99m}Tc-pertechnetate 1 mCi. Same to the JASB, incubation time was 10 minutes at room temperature. Labeled ^{99m}Tc-CSB was used coalescing material.

Energy and carbohydrate in JASB and CSB were calculated per that stated in Compendium of methods for food analysis (2003) p. 2 to 18 and p. 2 to 9. Moisture content was analyzed according to ASEAN Manual of Food Analysis (2011) p. 1 to 2 while ash, total fat, protein, total dietary fiber, and fructans (inulin + oligofructose) were analyzed according to AOAC (2012) 923.03, 922.06, 991.20, 985.29, and AOAC (2005) 997.08, respectively. Nutrient content and energy of JASB and CSB are given in Table 1.

Study design and protocol

JASB and CSB were examined by using a randomized, double-blinded, crossover design study. For a complete study, each participant was asked to visit the Division of Nuclear Medicine, Department of Radiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand twice with a one week wash-out period between each visit. Prior to each visit, the participant was required to fast for at least 6 hours before the examination. Upon arrival at the site, they were asked with some brief questions to ensure that they fully understood given instructions. They were then instructed to consume, depending on the visit, a JASB or a CSB (labelled with technetium-99m) followed by 250 ml of water within 10 minutes.

Gastric emptying study

Immediately after the completion of the JASB or the CSB consumption, individual participant was asked to position in front of the camera in an upright position at 45 degree left anterior oblique (45° LAO). The scintigraphic technique was then employed for gastric emptying analysis. A series of 45° LAO images, each of a 128x128 matrix, was acquired, dynamic 1 minute/frame images during the first 30 minutes and then statics images at 1, 1.5, 2, 3 and 4 hours, by using a gamma camera (Symbia Evo Excel, Siemens Healthcare GmbH, Erlangen, Germany; software: Syngo version. 10.100.1510.1604_VY21A08915), equipped with low energy all-purpose collimator.

Table 1. Nutrient Content and Energy of snack bars

	Snack bar (40 g)	
	JASB	CSB
Nutrients		
Carbohydrate (g)	24.9	24.1
Moisture content (g)	8.1	9.7
Ash (g)	1.0	0.6
Fat (g)	2.8	2.3
Protein (g)	3.2	3.4
Dietary fiber (g)	3.5	1.9
Fructans (Inulin + Oligofructose)	5.9	-*
Energy (kcal)	137.6	130.5

CSB = control snack bar; JASB = Jerusalem artichoke snack bar

* Fructans (inulin + oligofructose) in CSB were not analyzed

The analysis was performed on these scintigraphic images. Specifically, the gastric regions of interest [ROIs] were delineated closely around the entire stomach. The time-activity curve was then derived with radionuclide decay correction. The resultant fractional gastric retention curve was fitted with a modified exponential function, i.e., $y(t) = 1-(1-e^{-kt})^{\beta}$, where y (t) was fractional gastric retention at time t (minute), k was gastric emptying rate ($minute^{-1}$) and β was extrapolated y-intercept from the terminal portion of the curve. If the goodness-of-fit (R^2) between the fractional meal retention data and modified power exponential model was less than 0.9, only percent gastric retentions at 0.5, 1, 1.5, 2, 3 and 4 hours were calculated. Otherwise, the lag time and gastric emptying half time (GE $T_{1/2}$) were also calculated based on all obtained data by Couturier's formulae, i.e., given by $\ln \beta/k-(\beta-1)/k\beta$ and $-\ln(1-0.5^{1/\beta})/k$, respectively[23].

Satiety

In this study, subjective satiety was assessed by using 100-mm visual analogue scales [VAS][24]. Participants were ensured that they were familiar with VAS before commencing. Satiety (very full) and its opposing sensation (extremely hungry) were marked on either end of the 100-mm line. Each participant was asked how her felt and to what extent at that moment. They would respond to that question by marking a point on the line that best matched their feeling. The question and corresponding response were repeated at 0.5, 1, 1.5, 2, 3 and 4 hours. The satiety score was determined by measuring the distance from the left side (extremely hungry) of the line to the mark.

Statistical analysis

The sample size required for this study was determined based on 2-tailed; β = 0.05, power = 0.85, Cohen's d (effect size) was 0.80, which resulted in n = 29. Since this study was a randomized, double-blinded and crossover design, involving a comparison between the two groups, the n was thus set to 30, comprising of 15 participants per one group.All results were expressed as mean ± SEM, unless otherwise indicated. As previously stated, a non-linear regression analysis was performed on time-activity data points to calculate gastric emptying values; lag time, GE $T_{1/2}$, and percent gastric retentions. The associated satiety VAS scores were expressed as area under curve [AUC], which was in turn estimated using the trapezoidal numerical integral. The relationship between gastric emptying (percent retention) and satiety AUC were analyzed with independent sample t-test. Pearson's correlation coefficient was accordingly calculated with statistical significance of $p<0.05$. All statistical analyses were carried out using SPSS for Windows, Version 22 (licensed to Chulalongkorn University, Thailand).

Results

Radiochemistry

The first ^{99m}Tc-Jerusarem artichoke was successfully labeled using chelation. Silica gel instant thin-layer chromatography [ITLC] strips was used as a stationary phase and analysis in two solvent systems. Using acetone as mobile phase, the insoluble ^{99m}Tc-component was measured at the start (Rf = 0 to 0.1) and ^{99m}Tc-JASB and ^{99m}Tc-CSB were measured at Rf = 0.9 to 1.0. With 0.9 normal saline solution as mobile phase, free ^{99m}Tc-sodium pertechnetate moved with solvent front and was measured at Rf = 0.9 to 1.0 and ^{99m}Tc-JA and ^{99m}Tc-CSB were measured at Rf = 0.75. The radiochemistry purity of ^{99m}Tc-JASB and ^{99m}Tc-CSB were higher than 90% prior to baking.

Participants

There was no dropped out participants in the present study. All thirty healthy females had completed the required two visits to the hospital. The demographic, age and anthropometric (mean ages ±SEM, mean height ±SEM, mean weight ±SEM, and mean BMI ±SEM) are shown in Table 2.

Gastric emptying

Out of the total gastric emptying data analyzed, 91.67% could be fitted with modified power exponential function which the goodness-of-fit (R^2) greater than 0.9. Their descriptive statistics are listed in Table 3. An example of gastric emptying data and fitted curve using modified power exponential function

Table 2. Participant characteristics

	Participants (females)
Number	30
Ages (years)	29.1±2.0
Height (cm)	163.0±0.2
Weight (kg)	58.3±1.5
BMI (kg/m^2)	22.0±0.4

BMI = body mass index
Data were demonstrated as mean ± SEM

are shown in Figure 1.

The lag time represents the time for solid food to be triturated into small particles, which are then passed through the pylorus. The lag phase can be measured as the time from meal consumption to the first appearance of the radiolabeled solids in the proximal small bowel[25]. In this study, there was no statistically significant difference in lag time between consuming JASB and consuming CSB (p>0.05). Gastric emptying half time (GE $T_{1/2}$)is defined as the time required by the stomach to empty 50% of the ingested meal. It should be noted here that JASB samples exhibited significantly longer GE $T_{1/2}$ than did CSB ones (p<0.05) (Table 3).

It was further revealed that there were statistically significant differences between the percent gastric retentions of JASB and CSB (Table 3). Specifically, at 0.5, 1, 1.5, 2, 3, and 4 hours, percent gastric retentions of JASB were reported higher than those of CSB (p<0.05).

Satiety

AUC satiety scores at 0.5 and 1 hours of JASB did not differ from those of CSB (p>0.05). However, significant discrepancies were observed after 1.5, 2, 3, and 4 hours (p<0.05). Mean AUC of VAS scores for

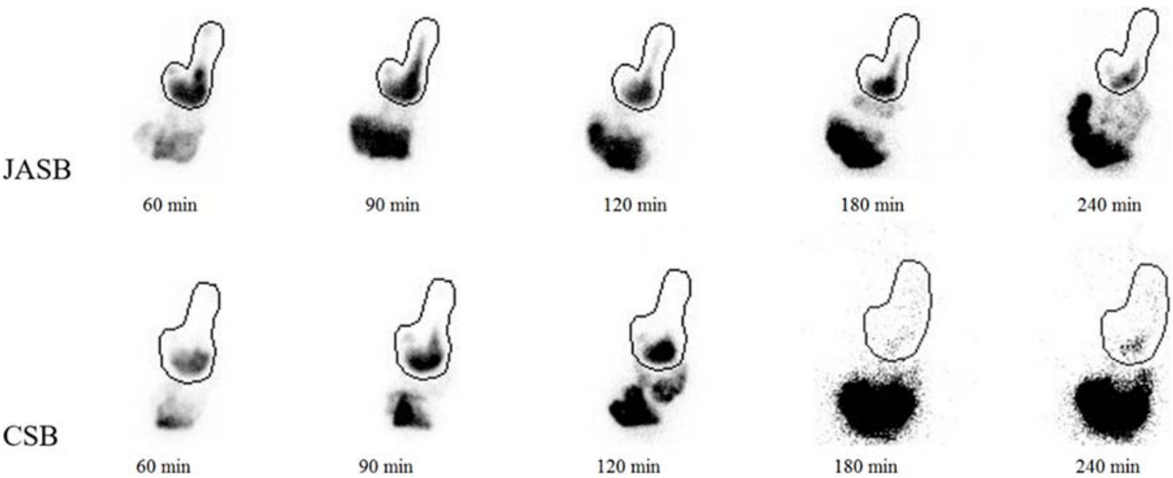


Figure 1. Examples of the gastric images and regions of interest [ROI] at different time points in one healthy female participant.

Table 3. Change of Gastric emptying values in the 30 healthy female participants

Gastric emptying values	JASB	CSB	Difference in mean (95% CI)	p-value
Lag time (min)	11.7±2.9	10.35±3.8	1.4 (-8.2, 11.0)	0.771
GE $T_{1/2}$ (min)	81.4±4.8	59.63±3.7	21.8 (9.8, 33.8)	0.001*
0.5 hour retention (%)	82.4±1.6	72.75±2.5	9.6 (3.7, 15.6)	0.002*
1 hour retention (%)	59.3±2.8	49.50±3.3	9.8 (1.2, 18.4)	0.026*
1.5 hour retention (%)	44.2±3.0	34.18±3.0	10.0 (1.5, 18.5)	0.021*
2 hour retention (%)	31.2±2.9	20.59±2.6	10.6 (2.8, 18.5)	0.009*
3 hour retention (%)	12.8±1.8	4.98±1.1	7.8 (3.6, 12.0)	0.001*
4 hour retention (%)	5.7±1.1	2.61±0.4	3.0 (0.7, 5.4)	0.012*

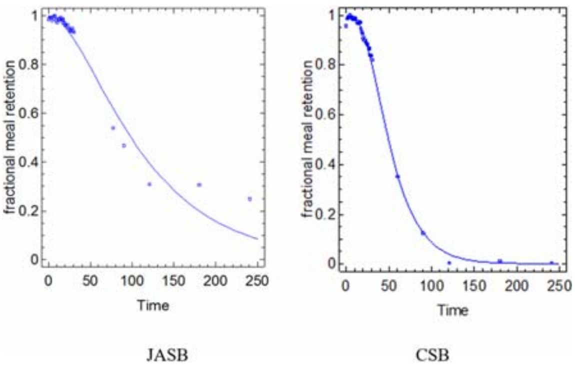
CI = confidence interval; CSB = control snack bar; GE $T_{1/2}$ = gastric emptying half time; JASB = Jerusalem artichoke snack bar

Data were demonstrated as mean ± SEM, * Significantly different from CSB (p<0.05)

Table 4. Change of AUC VAS Satiety in the 30 healthy female participants

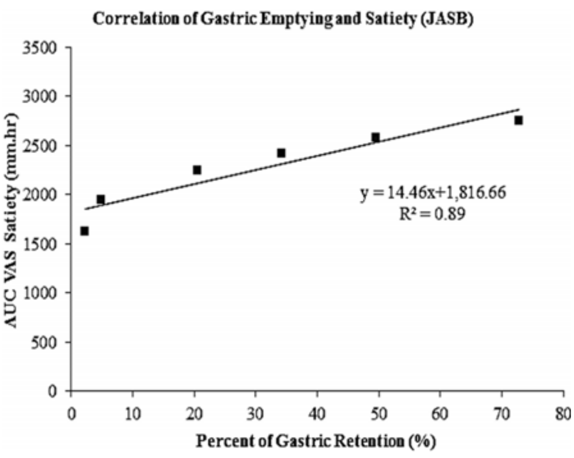
Time Points	AUC VAS Satiety (mm x hour)		Difference in mean (95% CI)	*p*-value
	JASB	CSB		
0.5 hour	2,755±37	2,648±52	107 (-20,234)	0.098
1 hour	2,575±43	2,434±59	141 (-6,288)	0.059
1.5 hours	2,418±53	2,236±59	182 (24,340)	0.025*
2 hours	2,249±70	1,980±71	269 (69,469)	0.009*
3 hours	1,947±93	1,591±102	357 (81,632)	0.012*
4 hours	1,622±112	1,186±117	436 (112,760)	0.009*

CI = confidence interval; CSB = control snack bar; JASB = Jerusalem artichoke snack bar
Data were demonstrated as mean ± SEM, * Significantly different from CSB ($p<0.05$)



CSB = control snack bar; JASB = Jerusalem artichoke snack bar

Figure 2. Fitted curve using modified power exponential function in one healthy female participant. Left panel is the curve derived from 99mTc-JASB and right panel is the curve derived from 99mTc-CSB with GE T1/2 of 61.2 and 46.7 min, respectively.



JASB = Jerusalem artichoke snack bar

Figure 3. Correlation of gastric emptying and AUC of VAS scores for satiety in JASB. The gastric emptying had a significant positive correlation with AUC of VAS scores for satiety ($R^2 = 0.89$, *$p<0.05$).

satiety are presented in Table 4.

Relation between gastric emptying and satiety in JASB

The relationship between gastric emptying and satiety in JASB was found to have a significant positive correlation ($R^2 = 0.89$, $p<0.05$) (Figure 3).

Discussion

On radiolabeling the inulin, utilizing $SnCl_2$ resulted in lower oxidation state of technetium-99m. In the presence of linear biopolymer of D-fructose units in inulin as suitable ligands, it easily chelates to form a stable ^{99m}Tc-JA complex. This had effectively enabled the tracing of JASB and CSB content after consumption.The same chelation methodology was previously reported in ^{99m}Tc-radiolabeled scrambled eggs which used as the ingested solid phase for gastric-emptying scintigraphy[25,26].

The results reported herein showed that JASB needed more time to be emptied from stomach and entering small intestine,effectively resulting in subjects' longer feeling of satiety. Being a component of edible plant cell, DF is classified as polysaccharide and generally is resistant to digestion in human digestive system. This is due to its slowing gastric emptying process and adding bulk to diet, contributing to gastric distension and fullness[13,27,28]. In this study, it is safe to say that JASB is a good source of fiber as it had 3.5 g DF which was higher than nominal 2.5 g[9]. In particular, the amount of DF in JASB was even higher than that of CSB (3.5 g versus 1.9 g). It is therefore rational to deduce that high DF concentration in JASB might play an important role in decreasing gastric emptying rate and as such prolonging fullness feeling in the participants. These findings were in concordance with that reported by Geliebter et al[27]. It their work, it was demonstrated that, between high-fiber oatmeal cereal and no-fiber corn flake cereal, the former much extended gastric emptying and gastric distention, then its counterpart and hence causing greater satiety[27]. Similar outcome was also evident in the study by Anderson et al (2009), which showed that meals containing pectin, soluble dietary fiber, could delay gastric emptying and enhanced satiety[29].

Satiety levels after having consumed a JASB at 1.5, 2, 3, and 4 hours were greater than those after consuming a CSB ($p<0.05$). This may have been due to slower gastric emptying effect and greater fiber content of JASB. This finding is in line with a previous work byYasmin et al in which whole grain pasta was compared with refined grain one. They established that the former had much fiber content and hence delayed gastric emptying and increased sensation of satiety. It was reasoned that the fiber content in whole grain pasta increased meal volume and viscosity of the stomach content[30]. Similar results were found by Slavin and Green, stating that DF increased chewing, which in turn limited food intake by promoting the secretion of saliva and gastric juice, and thus resulting in an expansion of the stomach and increased satiety[5]. Howarth, Saltzman, and Roberts indicated that foods that were high in DF were much satiating than low-fiber foods and could possibly decrease consequent hunger[8]. Ye et al showed that consuming DF may impact satiety by decreasing hunger, prolonging satiation, and/or increasing peripheral satiety signals[31]. Accordingly, it is reasonable to conclude that consuming high DF foods has notable impact on satiety due its ability to delay GE, effectively decreasing hunger and prolonging satiation.

JASB did not contain only DF but also inulin in its ingredients, i.e., dried JA tubers, JA flour, and JA syrup which contained inulin up to 50%, 60.66%, and 60%, respectively[16,32]. Since inulin cannot be digested in the upper gastrointestinal tract[33], when orally ingested, several inulin in JASB passed as small particles through the mouth and stomach without being digested. This effectively slowed the food transit, thus resulting in delayed gastric emptying and increased satiety. This finding was in accordance with that by Russo et al that, in young healthy subjects, a daily intake of 100 g of 11% inulin enriched pasta showed significantly prolonged gastric emptying in the treated group compared to the control one[34]. Moreover, Russo et al found that consuming inulin-enriched pasta affected the release of important gut peptides, neurotensin and somatostatin that involved in the control of gastric motor functions. This release resulted in notable delayed gastric emptying time. They also found that daily supplementation of 11 g inulin for 5 weeks could slow gastric emptying by 30 minutes compared with a control diet in healthy young men[35]. According to this study, inulin ingestion clearly has an effect on delaying gastric emptying. Inulin was found to also influence satiety in the study carried out by Singer et al in which coffee beverage enriched with inulin increased that feeling even 2 hours after ingestion[37]. Similarly regarding satiety feeling, Perrigue et al demonstrated that mixed low-energy-density yogurt beverages with inulin led to higher fullness ratings[38]. Therefore, inulin has advantages in both prolonging gastric emptying and increasing satiety.

The energy intake and the amount of fat, carbohydrate, and protein also play their parts in GE. Camps et al revealed that the energy load led to slower gastric emptying and higher amount of fat, carbohydrate, and protein could extend the GE rate[6]. Abell et al also showed that the emptying of fats was slower than emptying of proteins or carbohydrates[24]. Likewise, we conject that because JASB had much carbohydrate, fat, and energy than CSB (Table 1), it slowed down gastric emptying and thus making one feel satiety longer.

Conclusion

In this study, the first JA radiolabeling with ^{99m}Tc was successfully carried out to trace JASB in stomach and its effect on gastric emptying was evaluated. The radiochemistry purity of both ^{99m}Tc-JASB and ^{99m}Tc-CSB are more than 90% using instant thin-layer chromatography [ITLC]. JASB is a good source of DF and as such could prolong gastric emptying and increase satiety. Higher DF content in JASB (compared to CSB) played an important role in prolonging gastric emptyingand satiety. These results are relevant when considering using JASB as a primary material in weight control strategies. Further studies are however needed to examine the effect of JASB on the release of important gut peptides, in order to substantiate and better understanding of key contributing factors for satiety.

What is already known on this topic?

JA is known as functional food as an instigating agent of probiotic bacteria growth, resulting in increasing faecal biomass and water content of the stools. However, its role in gastric emptying as a satiety prolonging agent has remained yet to be evaluated.

What this study adds?

JA is a good source of inulin and DF and as such could plays a key part in delaying gastric emptying of consumed snack bar, resulting in prolonged subjects' satiety. Higher DF content in JASB (compared to CSB) played an important role in prolonging gastric emptying and satiety.

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Potential conflicts of interest

None.

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