JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

IN SILICO AND *IN VIVO* VALIDATION OF DIGESTION BEHAVIOR OF RICE IN THE ARTIFICIAL STOMACH ARK®

Sivakamasundari S. K., S. R. Priyadarshini, J. A. Moses, C. Anandharamakrishnan

Address(es): C. Anandharamakrishnan,
Computational Modeling and Nanoscale Processing Unit, Indian Institute of Food Processing Technology (IIFPT), Ministry of Food Processing Industries, Government of India, Thanjavur –613005, Tamil Nadu, India.

*Corresponding author: anandharamakrishnan@iifpt.edu.in

https://doi.org/10.55251/jmbfs.4392

ARTICLE INFO

Received 24. 2. 2021
Revised 28. 10. 2021
Accepted 13. 9. 2022
Published 1. 12. 2022

Regular article

OPEN ACCESS

ABSTRACT

The effect of the physicochemical properties on the digestion behavior of rice was evaluated using the artificial stomach response kit (ARK®). Due to the presence of the external bran layer, brown rice (Rv3) showed delayed rates of gastric emptying, apart from a higher half emptying time (101.80 ± 1.20 min) than milled rice (Rv1 - 57.84 ± 7.49 min) and basmati rice (Rv2 - 77.93 ± 10.18 min). The bran layer of Rv3 inhibited the diffusion of the simulated gastric fluid and resulted in lesser particle breakdown and low glycemic index (GI). In comparison with the shaker digestion process, the mechanical force produced in the ARK® resulted in a higher degree of particle breakdown. An *in silico* approach successfully predicted the glucose response pattern of rice varieties with no statistical difference (at $p < 0.05$) with the human *in vivo* datasets using the output obtained from ARK®. The GI obtained from ARK® was validated with *in vivo* data using Bland Altmann's statistical tool which showed good agreement. The morphology, dimensions, capacity of the stomach chamber in the ARK® also resembled *in vivo* observations. The ARK® is proposed as an improved alternative for *in vitro* digestion studies.

Keywords: Rice; artificial stomach; gastric emptying; in vitro digestion; glycemic index

INTRODUCTION

Food digestion is an intricate process with a significant research interest, particularly because of its role in human health and wellbeing. To develop specific food products with improved nutritional value, a strong understanding of the food digestion process is crucial. This includes digestion parameters like enzyme secretion, physical breakdown of food particles, transformation, and absorption of nutrients; understanding this bio-mechano-chemical process is challenging. Food digestion begins in the mouth with α-amylase, lingual lipase, and protease interactions. During mastication, foods get disintegrated, hydrated, and broken down, resulting in variations in bolus viscosity **(Sethupathy, Moses, & Anandharamakrishnan, 2020)**. After mastication, the food undergoes gastric digestion in the stomach.The human stomach consists of two regions, proximal(fundus and corpus) and distal(antrum and pylorus) **(Gopirajah & Anandharamakrishnan, 2016)**. Enzymatic digestion occurs in the fundus where the food is stored and peristaltic movement mixes and breaks down the food in the antrum. At the end of the gastric digestion process (with particles reaching <2 mm), the digesta empties from the stomach through the pyloric valve to the small intestine. Several parameters decide the disintegration and the emptying rate of different food matrices. As these parameters decide the release and absorption of nutrients, it is important to know their relationships **(Gopirajah & Anandharamakrishnan, 2016)**.

Rice is the principal source of carbohydrate and energy in the south Asian diet and is consumed in value-added forms. The digestion behavior of rice is influenced by varietal differences, postharvest processing conditions, cooking methods, and storage practices, among others **(Sivakamasundari, Priyanga, Moses, & Anandharamakrishnan, 2020)**. Over the years, there has been a growing interest in simulating the digestion process of the gastrointestinal tract, attempting to best relate it to the *in vivo* process. However, most *in vitro* methods do not accurately reproduce the physiological process of digestion. For instance, the mechanism of gastric emptying is often not well accounted though it has a pronounced role in nutrient absorption in the small intestine. Another limitation is the lack of consideration of the mechanical forces of an actual digestion process. Given these concerns, various dynamic gastric models have experimented with; several of these do not consider the morphological and anatomical features of the human gastrointestinal system, though these are important factors that influence the rate of digestion and absorption. The predictive *in silico* approach has recently been identified as an interesting technology particularly in relation to the immense clinical datasets and this approach can be deployed to predict glucose responses of food especially for individuals with type 2 diabetes.

In this research, we explain the impact of the physicochemical properties of rice on its digestion behavior. Different rice varieties were digested using an artificial stomach response kit (ARK®) and the results were compared with the conventional static *in vitro* digestion process. The digestion behavior was explained through a series of parameters and the glycemic index (GI) of the rice varieties was studied. Attempts have been made for predicting the glucose responses for the rice varieties using a predictive *in silico* algorithm considering some of the digestive parameters obtained from ARK®.

MATERIALS AND METHODS

Selection of rice variety

In this study, rice varieties with varying physical structure and amylose content were selected. These include low amylose rice (var. ponni -milled rice; Rv1), high amylose rice (var. basmati; Rv2), and brown rice with bran (var. ponni; Rv3). All chemicals and reagents used in the study were of analytical grade.

Physiochemical properties of rice

The physicochemical properties of rice were determined based on the procedure adopted by **Sivakamasundari, Moses, & Anandharamakrishnan, (2020)**, and fiber content (%) was measured by the AOAC method (**AOAC, 2016**).

Sample preparation

The conventional boiling method was used to cook the rice samples and their hardness was determined using a texture analyzer (Stable Micro Systems, TA-XT2i, Surrey, UK). Cooked rice was placed as a single layer on the base plate of the texture analyzer; a 35 mm cylindrical probe was used for a two-cycle compression test with a pretest speed of 0.5 mm/s, test speed of 1 mm/s, and strain of 80%. The maximum compression force in the first cycle was recorded as the hardness of rice. Apart from that, cooked rice properties were determined using the method reported by **Sivakamasundari *et al.*, (2020)**.

Dynamic digestion model ARK®

In this study, the digestive behavior of the different rice varieties was determined using the ARK® (Figure 1). It consists of a stomach chamber seated on a stainless steel base plate; the 3D-printed stomach was designed to replicate the geometry and capacity of the human stomach as evaluated through abdominal MRI scans of

human volunteers **(Gopirajah, Raichurkar, Wadhwa, & Anandharamakrishnan, 2016)**. The"J" shaped geometry of the stomach can better reproduce the gastric distribution and emptying patterns of foods **(Li, Yu, Wu, & Chen, 2020)**. The ARK® is fitted with pneumatic piston arrangements on the base plate at three distinct positions covering the stomach area, to provide the required mechanical force to the food by squeezing the stomach wall around the antrum region. The pneumatic piston arrangements were connected to a solenoid valve that allowed contractions at every 18 – 20 s interval. The frequency and depth of contractions on the stomach chamber were controlled using a programmable logic controller unit (PLC) and computer. The rate of emptying of foods from the ARK® relies on the pressure in the gastro-duodenal region provided by the pneumatic piston arrangements. In the lower part of the stomach, a wire mesh of size 2 mm was placed thus it reproduces the sieving effect of pyloric region **(Ranganathan, Vasikaran, Elumalai, Moses, & Anandharamakrishnan, 2021)**. Also, the temperature and pH in the ARK® were controlled using an electric lamp and a pH-stat, respectively.

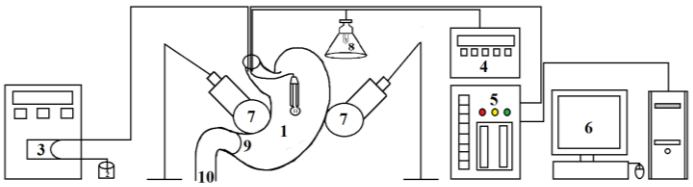

Figure 1 Schematic representation of ARK®
1. Stomach chamber, 2. Simulated gastric fluid, 3. Peristaltic pump, 4. pHstat, 5. PLC unit, 6. PLC unit connected to the computer, 7. Pneumatic piston set up, 8. Electric bulb to maintain temperature, 9. Mesh that creates a sieving effect (diameter 2 mm), 10. The outlet for gastric digesta collected using a peristaltic pump, 11. pH probe

Dynamic *in vitro* digestion of rice

Around 150 g of cooked rice was homogenized using a mortar and pestle and incubated with salivary enzymes (Sodium chloride, Hi-Media; potassium chloride, Hi-media; sodium bicarbonate, Hi-Media;α-amylase, Sigma; mucin, Sigma; pH~7).Then, the homogenized samples were transferred to the ARK® for gastric digestion that contained preloaded (10 mL) simulated gastric fluid (SGF) to imitate fasting conditions. Additionally, SGF (pepsin, Sigma; mucin, Sigma; sodium chloride) was continuously added to the sample using a peristaltic pump (Parisa Technology, Mumbai, India) **(Kong, Oztop, Singh, & McCarthy, 2011)**. The emptying of gastric contents from the stomach chamber after digestion was facilitated using another peristaltic pump (2.5 mL/min) attached near the outlet of the pyloric valve. During the experiment, the pH was maintained in the range of 1.5 - 3 using a pH-stat (Spectra lab, AT 38C, Mumbai, India). Similar to the dynamic digestion (in ARK®); rice samples were digested in a shaker reciprocating at 100 rpm at 37˚ C. In both cases, the digesta was collected to determine its characteristics.

Determination of flow rate, pH, and absorption of SGF during dynamic *in vitro* digestion

To replicate *in vivo* gastric secretions, the flow rate (mL/min) of the SGF added into the ARK® was measured every 15 min for 2 h, and the resulting changes in the pH of the digesta from the outlet were monitored using a pH probe. During digestion, the absorption of SGF by the rice samples retained in the stomach chamber was also measured by calculating its moisture content (%wet basis; 30 min interval for 2 h).

Morphology of rice after digestion

The morphological changes of rice before and after digestion were observed using a stereomicroscope at 20X magnification (Motic* K Series Stereo, Model K400 L Leica Microsystems Ltd., Wetzlar, Germany).

Particle breakdown of rice

Initially, after the oral phase of digestion, the particle size distribution of the rice samples was determined using sieve analysis (sieve size: 4 mm, 2 mm, 1 mm, 0.71 mm, and 0.56 mm). Further,to determine the rate of particle breakdown of rice under both *in vitro* digestion conditions, undigested particles from the stomach chamber were collected every 30 min for 2 h and placed in a Petri dish with water to identify individual particles. A digital camera was used to capture images, and the particle size (mm) was determined using Image J software **(Sethupathy, Moses, *et al*., 2020)**.

Modeling the disintegration rate of rice

The rate of disintegration of solid foods from the stomach is directly related to the time of gastric emptying and was therefore determined for the rice samples from the mass retention ratio (30 min interval for 2 h) using Eq.(1). The mass of rice retained in ARK® at time t (wt) was determined by emptying the stomach contents and recording its weight (g). The mass retention ratio data weret then fitted with Siegel's modified power exponential equation Eq. (2) using Microsoft Office Excel 2007 Solver to determine the rate of disintegration of rice particles. Also, $t_{1/2}$ (half time emptying of solids from the stomach chamber, min) was calculated from Eq. (3) **(Kong & Singh, 2008b).**

$$y_t = \frac{W_t}{W_0} \quad \text{Eq.1}$$

$$y_t = 1 - (1 - e^{-kt})\text{^}\beta \quad \text{Eq.2}$$

$$t_{\frac{1}{2}} = \left(\frac{-1}{k}\right) * \ln(1 - 0.5^{\frac{1}{\beta}}) \quad \text{Eq.3}$$

Where;

y_t	=	Mass retention ratio (g/g);
W_0	=	Mass of rice before digestion (g);
W_t	=	Mass of rice (g) retained in the ARK® at time t;
k	=	Emptying rate of food (1/min);
β	=	Shape factor or y intercept extrapolated from the curve.

Prediction of glucose responses by *in silico* algorithm

An *in silico* analytical approach was formulated to predict the glucose responses for all the rice varieties. An algorithm was developed in MATLAB (R2019a), Math works® Natick, USA, to capture the dynamics of glycemic relevant variables. Here, the linear regression relationship was established between the response and predictors where the linearity indicates the dependency of predictor coefficients. The machine was allowed to predict the glucose response trend using baseline variables (particle size and pH) upon different rice varieties based on least squares fit. The general model is shown in Eq. 4. The model was then validated with the human *in vivo* glucose response.The *in-vivo* blood glucose response for the rice varieties was performed in compliance with the International GI research criteria **(International Standard Organization, 2010)**. The *in vivo* human studies were authorized under 122DD of the Drugs and Cosmetics Rules, 1945, by the Institutional Ethics Committee (Registration No: ECR/946/Inst/TN/2017) of Meenakshi Hospitals, Thanjavur. With 10 participants (age group 25 - 30) for three separate rice varieties, the *in vivo* research protocol was performed. Subjects with insulin impairment, implants, significant surgery, nursing women, and people with digestive issues were the restriction criteria. Written permission was received to carry out the study from all the participants. Also, the study was approved by the institutional review board.

$$y \sim 1 + x_1 + {x_1}^2 \quad \text{Eq. 4}$$

Where;
1 – Intercept,
x_1, ${x_1}^2$ - linear and squared terms for each predictors.

Determination of glycemic index of rice*in vitro* and its comparison with *in vivo*

Simulated gastrointestinal digestion of rice was performed with slight modifications to the protocol given by **Ye *et al*., (2019)**. Cooked rice with 50 g available carbohydrates was taken, and to this, 10 mL of simulated salivary fluid (SSF) was added for oral digestion (SSF: α-amylase, Sigma - ≥ 5units/mg solid in carbonate buffer; pH 6.8 for 1 min at 37˚C). After oral digestion, the samples were transferred to the ARK® for gastric digestion; SGF (pepsin, Sigma - ≥ 250 units/mg solid in 0.02 Mhydrochloric acid) was added accordingly and the pH was maintained in the range of 1.5 – 3 until 1 h. After gastric digestion, the digestaemptied through the mesh was collected and its pH was adjusted to 7 immediately using 2 M sodium hydroxide. Further, 20 mL simulated intestinal fluid (SIF; pancreatin from porcine pancreas, Sigma - 8 x USP specifications, and amyloglucosidase from *Aspergillus niger*, Sigma - 260 units/mL in acetate buffer) was added to the emptied digesta. The samples were then digested in a shaker incubator at 37˚ C for 120 min, 100 rpm. GODPOD kit (Glucose oxidase-peroxidase kit, Baecon, India) was used to determine the release of glucose at 0, 20, 30, 60, 90, and 120 min from the digested samples **(Sethupathy, Sivakamasundari, Moses, & Anandharamakrishnan, 2020)**.
From the glucose release, the starch hydrolysis (%) of rice was determined. Also, the hydrolysis index (HI) and the estimated glycemic index (eGI) of rice were determined by Eq. (5) and (6). Glucose (HI Media, MB037) was used as the reference food **(Santhi Rajkumar, Suriyamoorthy, Moses, & Anandharamakrishnan, 2020)**. Further, the *in vitro* GI of three different rice varieties obtained from ARK® was compared with *in vivo* **(Arvidsson-lenner *et al*., 2004)**.

$$HI = \left(\frac{AUC_{sample}}{AUC_{reference\ \ food}}\right) \times 100 \quad \text{Eq.5}$$

$$eGI = 39.71 + (0.549 \times HI) \quad \text{Eq.6}$$

Where;
AUC – Area under the curve.

Statistical analysis

All experiments were performed in triplicates and the mean and standard deviation values were calculated. SPPSS software (ver. 20.0) was used to analyze significant differences among the samples by one-way ANOVA at $p<0.05$. Regression analysis was performed using Origin Pro software (ver. 8.0). Correlation between *in vitro* and *in vivo* GI was determined using the Bland-Altmann method in Graph Pad Prism software (ver. 8.4.3) **(Priyadarshini, Arunkumar, Moses, & Anandharamakrishnan, 2021)**.

RESULTS AND DISCUSSION

Physicochemical properties of rice

The physicochemical properties of rice were significantly different for each variety at $p<0.05$. Rv3 had higher breadth (2.56 ± 0.10 mm) and thickness (1.86 ± 0.09 mm) than Rv1 (breadth: 1.79 ± 0.16 mm;thickness: 1.43 ± 0.07 mm) and Rv2 (breadth: 1.58 ± 0.12 mm;thickness: 1.42 ± 0.06 mm). Whereas, Rv2 had a higher length (Rv1: 4.87 ± 0.33 mm; Rv2: 7.45 ± 0.76 mm and Rv3: 5.78 ± 0.24 mm). The sphericity was higher for Rv3 (52.30 ± 1.66%) than Rv1 (47.70 ± 2.44%) and Rv2 (34.53 ± 2.97%). The 1000 kernel weight for the rice varieties was 11.64 ± 0.89 g for Rv1, 15.05 ± 0.06 g for Rv2, and 21.03 ± 0.46 g for Rv3, respectively. The moisture content of all rice varieties ranged between 9.41% – 11.23% wet basis.
The fiber content of Rv3 was significantly ($p<0.05$) higher (5.12 ± 1.56%) than Rv1 (0.82 ± 0.12%) and Rv2 (1.36 ± 1.05%). In addition, Rv2 had significantly ($p<0.05$) higher amylose content (28.42 ± 1.66%) than Rv1 (21.65 ± 2.56%) and Rv3 (22.35 ± 0.39%); Rv3 (72.43 ± 0.56%) and Rv2 (71.56 ± 1.26%) had lower starch content than Rv1 (77.65 ± 1.58%).
The water absorption ratio (Rv1: 3.82 ± 0.06; Rv2: 3.12 ± 0.08 and Rv3: 5.23 ± 0.12) and cooking time (Rv1: 28 ± 1.58 min; Rv2: 20.30 ± 0.56 min and Rv3: 50.26 ± 2.38 min) was higher for Rv3, with reverse trends in the case of solid loss (Rv1: 5.63 ± 0.56%; Rv2: 4.70 ± 0.45% and Rv3: 2.16 ± 0.40%). The hardness of cooked rice varied significantly ($p<0.05$) between the varieties (with 717.13 ± 95.26 g for Rv1; 866.33 ± 86.25 g for Rv2 and 795.97 ± 102.86 g for Rv3). With a reduction in solid loss, the hardness of cooked rice was higher.

Flow rate and pH of SGF during digestion in ARK®

The flow rate of SGF added into ARK® during digestion was maintained around 2.72 ± 0.06 mL/min using a peristaltic pump, similar to the *in vivo* observations recorded by **Malagelada, Go, & Summerskill, (1979)**. Likewise, in a recent advanced near-real dynamic *in vitro* human stomach system, the average flow rate of SGF was around 2.9 mL/min **(Wang *et al*., 2019)**. The pH profile of the gastric digesta was also recorded (Figure 2) where, the initial pH of SGF was ~ 1.5 but increased rapidly after adding rice in the ARK®, irrespective of the varieties. This was because, when rice was added after oral digestion, its buffering effect raises the pH; however, pH gradually decreases after the continuous addition of SGF. Moreover, after oral processing, rice starch gets hydrolyzed by the salivary enzyme α-amylase **(Mennah-govela, Bornhorst, & Singh, 2015)**, further increasing the acid diffusion rate, resulting in a reduced pH. Rv3 had a significantly higher pH profile than Rv1 and Rv2, indicating that the diffusion of SGF inside the kernel might be difficult due to the presence of the bran layer. Rv2 also had a higher pH profile due to its higher amylose content, in turn, decreasing the diffusion of SGF inside the kernel; amylose can be retrograded easily after cooking thus preventing the diffusion of SGF during digestion **(Syahariza, Sar, Hasjim, Tizzotti, & Gilbert, 2013)**. The pH profile of the rice varieties digested in ARK® was compared with digestion in a shaker; for all the rice varieties, the pH did not vary significantly (Figure 2) and **Liu *et al*., (2019)** reported similar results for protein digestion in static, semi-dynamic and artificial gastric digestion methods.
Thus, the matrix of any food can have a noticeable impact on the diffusion of gastric fluids. Using studies on carrots **(Van Wey *et al*., 2014)** explained that gastric fluid diffusion was pH-dependent, and a lower solid loss is associated with a higher pH. Thus Rv2 and Rv3 showed a lower solid loss during digestion with associated delayed gastric emptying.

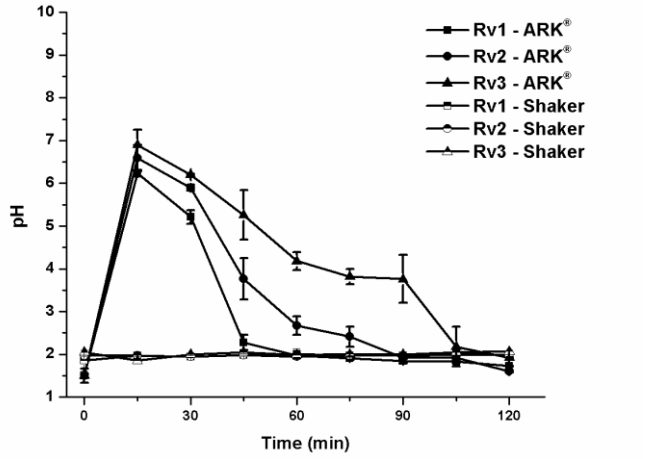

Figure 2 The pH profile of digesta during gastric digestion

Moisture absorption of rice during digestion

The absorption of SGF by the rice samples (hydration behavior) during digestion in the ARK® was measured (Figure 3); Rv3 showed lower absorption due to the presence of the outer bran layer. It was reported by **Horigane, Takahashi, Maruyama, Ohtsubo, & Yoshida, (2006)** that the bran layer prevents water diffusion; so, much of the absorption occurred near the periphery of the embryo, but moisture diffusion in white rice occurred through the surface cracks. The results are also consistent with observations in the pH study. The hardness of cooked rice was found to be inversely related to its SGF absorption, and Rv3 with higher hardness absorbed lesser SGF whereas; these values were directly correlated with solid loss during cooking. In the case of Rv2, higher amylose content during cooking tends to form an amylose lipid complex that prevents starch from swelling and thus making it resistant to SGF absorption **(Hasjim, Ai, & Jane, 2013)**. Also, the lower amylose content of Rv1 may increase its SGF absorption, thus improving digestibility.
Rice digestion in the ARK® was also compared with digestion in the shaker and the results are shown in Figure 3. Digestion methods showed significant differences; more SGF absorption was found for the rice digested in ARK® than the shaker. This was because the mechanical force created in the shaker might not be sufficient to transfer the SGF to the kernels, but the pneumatic piston arrangements in the ARK® could facilitate the transport of moisture by providing the mechanical force required for particle breakdown by overcoming its internal resistance. Similar observations were reported by **Kong *et al*., (2011)**.

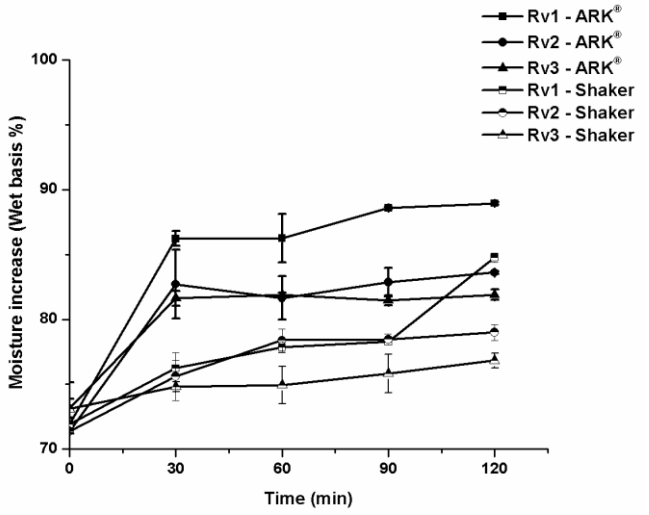

Figure 3 Moisture absorption of rice during gastric digestion

Morphology of rice before and after digestion

Before and after digestion in ARK®, the microstructure of rice samples (Figure 4, 1 - 6) was determined to examine the effect of digestion on the structural variations of rice varieties. It wasobserved that erosion of solids from the surface had occurred in the case of Rv1 and Rv2 whereas; the presence of the bran layer on the kernel was evident even after digestionin the case of Rv3. Besides, during cooking, Rv3 showed higher water absorption and lower solid loss due to the presence of

the bran layer. **Kong *et al*., (2011)** also observed that the presence of the bran layer in brown rice slowed its gastric emptying rates. Thus, in Rv3, the bran layer delayed digestion by preventing the permeation of SGF.

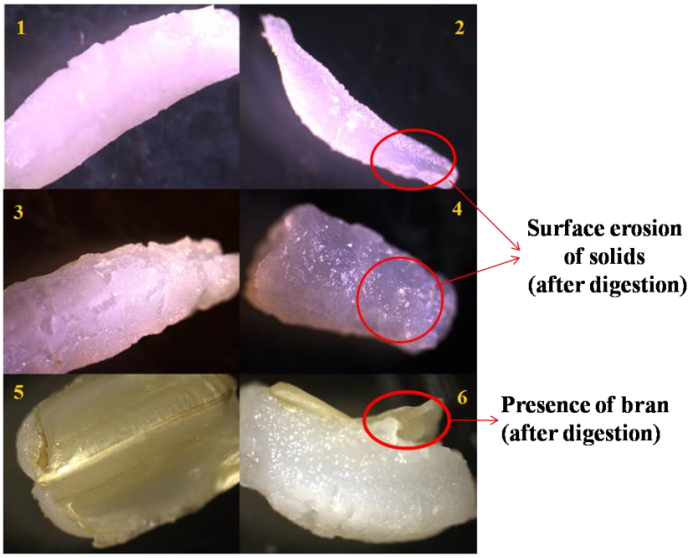

Figure 4 The morphological changes of rice before and after gastric digestion in the ARK®

Particle breakdown of rice during digestion

The particle size of rice retained in ARK® and shaker during digestion is shown in figure 5. At 0th min, the particle size of Rv3 was larger than that of Rv1 and Rv2 (Rv1: 32.95%, Rv2: 34.36%; Rv3: 45.36% particles were greater than 4mm) (Figure 5a), though it was homogenized during the oral digestion. The higher particle size (as in the case of Rv3) can extend the time for digestion and hence lower gastric emptying rates (Tab 1). It was evident that the particle size of rice retained in the ARK®decreased as the digestion proceeds; Rv1 showed an average particle size of 3.26 mm while for Rv2 and Rv3 these values were 3.63 mm and 3.8 mm, respectively (at 120 min). On the contrary, rice digestion in the shaker did not decrease the particle size considerably (Figure 5b). For the first 1 h, the particle size was almost similar for both the digestion methods, but large particles were observed in the shaker digestion even at the end of digestion irrespective of the varieties (Rv1: 4.05 mm, Rv2: 4.76 mm, Rv3: 5.95 mm) and these observations were significantly different from digestion in ARK® (p<0.05). The findings are in line with rice moisture absorption; digestion in ARK® displayed more absorption of SGF than the shaker digestion. Further, the higher hardness of Rv3 with higher cooking time and lesser solid loss during cooking resulted in significantly higher particle size during digestion. Also, from the linear regression analysis, a higher positive correlation (R^2>0.90) between the time and particle breakdown during gastric digestion was observed for all varieties irrespective of the digestion methods. Thus, digestion in the shaker did not provide the mechanical force to the particle breakdown despite it being an important parameter that influences the chemical digestion of food in the stomach and subsequent nutrient absorption in the small intestine.

Modeling disintegration kinetics

The breakdown of food particles in the stomach occurs due to erosion, fragmentation, and tenderization. Erosion of food particles occurs when the applied force during digestion is lesser than that required to create a fracture. Fragmentation refers to the breakdown of food particles into two or more pieces, and the penetration of gastric fluid into the food particles during tenderization softens its texture **(Ferrua, Kong, & Singh, 2011)**.

The parameters for disintegration kinetics of rice during digestion in ARK® were calculated from the modified power exponential equation and are given in tab 1. The rate of gastric emptying for Rv1 (0.01 min^{-1}) and Rv2 (0.01 min^{-1}) were higher than that of Rv3 (0.005 min^{-1}) and this can be attributed to the bran layer that requires more digestion. **Bornhorst, Chang, Rutherfurd, Moughan, & Singh, (2013)** observed similar results during *in vivo* digestion of white and brown rice.

For the rice varieties, β was greater than 1 due to the delayed emptying of solids during the initial digestion stages **(Kong & Singh, 2008a)**. Besides, the $t_{1/2}$ for Rv1 (57.84 ± 7.49 min) was significantly (p<0.05) lower than Rv2 (77.93 ± 10.18 min) and Rv3 (101.80 ± 1.20 min) explaining that the physicochemical properties of rice can alter its gastric emptying rate. **Pletsch & Hamaker, (2018)** observed delayed gastric emptying rate and half time emptying ($t_{1/2}$) for brown rice than white rice due to two major reasons: (i) The physical structure of brown rice with an extra bran layer requires a longer time to break down particles, (ii) broken particles require a longer time to digest than white rice, resulting in slower digestion. Further, rice with higher amylose content had a higher viscosity after oral digestion **(Kim, Oh, Kim, & Lee, 2019)** and thus Rv2 had greater $t_{1/2}$ than Rv1. These results are consistent with the particle breakdown values in the ARK® (Figure 5b). Also, the $t_{1/2}$for Rv1 and Rv2 were in line with the results reported in other *in vitro* **(Wang *et al*., 2019)** and *in vivo* studies **(Mano *et al*., 2018)**.

The gastric retention of solids (%) (Undigested solids) in the ARK® was measured after 2 h and shown in tab 1. Similar to the above results, a higher percentage of gastric retention was observed for Rv3 (statistically insignificant, p>0.05) than for Rv1 and Rv2 as particles greater than 2 mm size get retained in the stomach for further breakdown **(Bornhorst, Kostlan, & Singh, 2013)**. This can also be an explanation for Rv3's delayed gastric emptying rate and higher $t_{1/2}$. A reduced but positive correlation (R^2 = 0.51) between solid retention and $t_{1/2}$ was observed for rice during digestion irrespective of the varieties. These results were further confirmed with moisture absorption, particle breakdown, and microstructure analysis; these explained that Rv3 underwent lesser particle breakdown and lesser absorption of SGF.

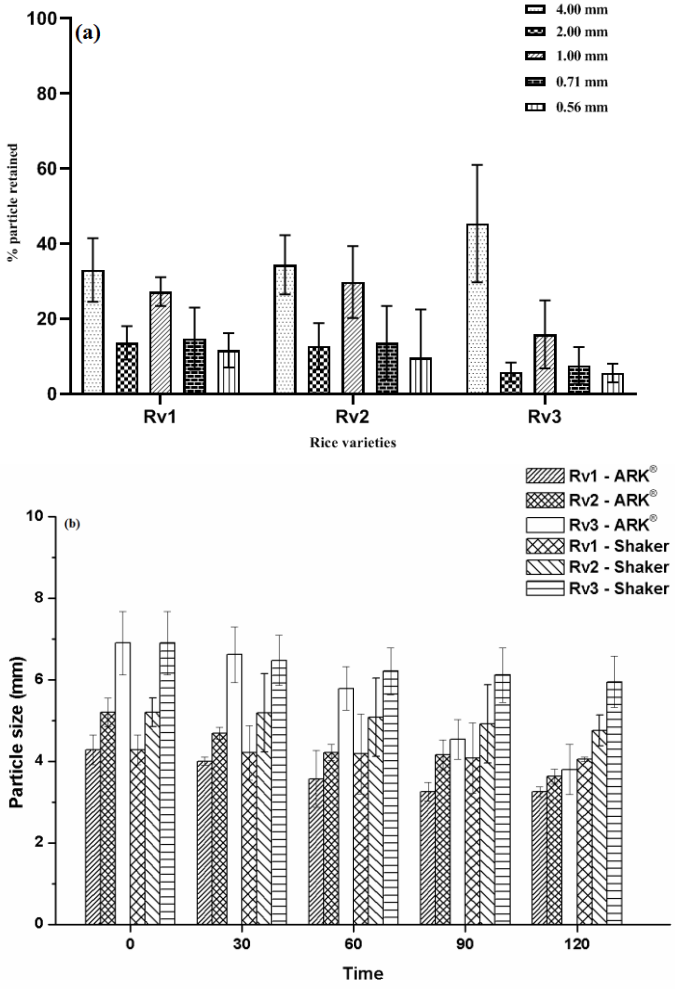

Figure 5 The particle size distribution of rice during oral phase of digestion (a); the particle size of rice during gastric digestionat different time intervals in the ARK® (b)

Table 1 The disintegration of rice during gastric digestion in ARK®, its kinetic parameters**,** *in vitro* starch hydrolysis, and estimated glycemic index (*in vitro* and *in vivo*)

Disintegration kinetics parameters of rice digested in ARK®						**Starch hydrolysis and GI of rice during gastric digestion in ARK®**					**In vivo GI**	
Sample	**Solid retention (%)**	**Gastric emptying rate (min^{-1})**	**β**	**$t_{1/2}$ (min)**	**R^2**	**C_∞ (%)**	**k (min^{-1})**	**HI**	**eGI**	**R^2**	***i*GI**	
Rv1	33.00 ± 12.9[a]	0.01 ± 0.000764[a]	1.16 ± 0.04[a]	57.84 ± 7.49[a]	0.99	70.88 ± 5.53[a]	0.07 ± 0.01[a]	61.54 ± 5.01[a]	73.50 ± 2.75[a]	0.99	77.49 ± 2.35[a]	
Rv2	48.00 ± 8.50[a]	0.01 ± 0.001732[a]	1.10 ± 0.08[a]	77.93 ± 10.18[b]	0.97	59.93 ± 0.09[b]	0.09 ± 0.01[a]	53.40 ± 0.54[ab]	69.03 ± 0.30[ab]	0.99	74.66 ± 2.15[a]	
Rv3	49.00 ± 9.07[a]	0.005 ± 0.000115[a]	1.27 ± 0.01[ab]	101.80 ± 1.20[c]	0.97	61.92 ± 2.94[ab]	0.05 ± 0.02[ab]	48.02 ± 3.08[b]	66.08 ± 1.69[b]	0.97	69.70 ± 4.96[b]	

β – shape factor or y-intercept extrapolated from the curve; $t_{1/2}$ – Half time emptying (min); C_∞ - Equilibrium percentage of hydrolyzed starch; k –Starch hydrolysis rate (min^{-1}); HI – Hydrolysis index; eGI – Estimated glycemic index. iGI – In vivo glycemic index.
Different alphabets in the superscript of columns indicate a statistically significant difference (at p <0 .05) between the rice varieties

In silico approach to predict glucose response pattern of rice

The *in vivo* glucose response pattern of each rice variety was determined from the predictive *in silico* algorithm. The statistical performance (*p*-value) of each model on each rice variety was validated with the *in vivo* datasets. The results as summarized in tab 2 showed no significant differences (at $p>0.05$) between the results obtained from the *in silico* approach and the *in vivo* datasets establishing a strong simulation. All the results showed a peak at 30 min and thereafter declined, where the predicted glucose release pattern followed the same trend as the human datasets. When particle size and pH values are obtained directly from the ARK® system, this predictive model can be used to predict the glucose responses of rice varieties, without performing human blood sampling. It is a well known fact that the glucose response of foods varies with particle size **(Mackie *et al*., 2017)** and the pH balance in the stomach varies based on food nutrient content ultimately bringing differences in the glycemic responses **(Stacher, Bauer, Schulze, Pointner, & Landgraf, 1976)**. Therefore, the changes in particle size and pH are good predictors of glycemic responses of food.

Table 2 Predicted glucose response using predictive *in silico* algorithm and its comparison with human *in-vivo* data

Time (min)	**Rv1**			**Rv2**			**Rv3**		
	***p*GR**	***i*GR**	***p*-value**	***p*GR**	***i*GR**	***p*-value**	***p*GR**	***i*GR**	***p*-value**
0	104.19	93.00±8.19	0.1996	102.07	96.40±6.80	0.4033	95.10	97.20±6.72	0.9666
30	128.14	131.20±15.19	0.5791	125.33	128.20±3.96	0.9636	117.24	118.20±17.38	0.9883
60	103.24	116.80±1.30	0.0988	102.92	109.00±4.95	0.2772	101.83	101.00±15.60	0.9143
90	101.83	102.00±8.57	0.9749	99.51	98.60±3.65	0.9475	94.71	91.60±9.53	0.7063
120	101.49	95.20±9.42	0.4364	96.48	93.00±8.92	0.6273	86.49	87.20±7.66	0.7219

Values of in-vivo data are represented as mean ± SD (n=10) pGR: predicted glucose response; iGR: in vivo glucose response

Glycemic index of rice and its correlation with *in vivo*

The hydrolysis of the rice starch is given in tab 1. Distinct variations can be seen in the rates of starch hydrolysis (k min^{-1}) between the varieties; Rv2 and Rv3 had lower starch hydrolysis rates than Rv1. Also, for Rv2 and Rv3, C_∞ (equilibrium percentage of hydrolyzed starch) was lower (Tab 1). The HI and eGI were determined and Rv1 had a GI of 73.50, while Rv2 and Rv3 had lower GI values of 69.03 and 66.08, respectively. Moreover, the GI of rice in this study was negatively correlated with the hardness of cooked rice and solid loss during cooking; whereas it was directlyrelated to the moisture absorption and particle breakdown during digestion. Cooking time was higher for brown rice, similar to the observations of **Ruchi, Mohan, Ramya Bai, & Sudha, (2014)** in which brown rice had low GI.
GI may also be influenced by the rate of gastric emptying of solids; Rv3 had a lower rate of gastric emptying and higher $t_{1/2}$ (Tab 1), resulting in low GI as the bran layer in the brown rice consists of non-starch polysaccharides that act as a barrier to enzymatic hydrolysis **(Shobana *et al*., 2017)**. Also, the presence of phenolic compounds in the bran layer of brown rice may contribute to partial digestive enzyme inhibition **(Mohan *et al*., 2017)**. Besides, the bran layer could act as a physical barrier during digestion, preventing the entry of digestive enzymes and swelling of starch granules during cooking and digestion. Similar observations were reported by various researchers. **Somaratne *et al*., (2017)** reported a negative relationship between fiber content and GI of rice, and similarly, Rv3 with higher fiber content had the lowest GI. Therefore, the consumption of brown rice can delay gastric emptying and reduce GI. Apart from that, for all rice varieties, a high positive correlation (R^2 = 0.94) was observed between $t_{1/2}$ and GI. Thus, in determining the GI of foods, the gastric emptying rate of solids should be considered as it has a significant impact. Following Rv3, higher amylose content and $t_{1/2}$ of Rv2 contributes to its lower GI due to the formation of amylose lipid complex during cooking which may also delay the starch hydrolysis. Also, after cooking, amylose gets easily staled and digested similarly to dietary fiber **(Chang, Hong, Jung, & Suh, 2014)**; thus, Rv2 had low GI than Rv1.
The GI results obtained from ARK® were then compared with *in vivo* GI and given in tab 1. From Bland Altmann's statistical test (Figure 6), it can be observed that the results were found to agree with the upper and lower limits respectively. Thus, the ARK® (considering digestion parameters such as the pH of digesta, the flow rate of SGF, effective particle breakdown, gastric emptying) showed results similar to *in vivo* glycemic response and thus it can be effectively used for determining the GI of foods.

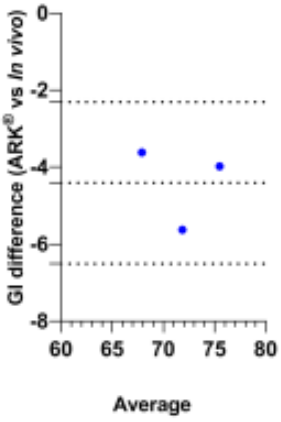

Figure 6 Validation between *in vitro* (ARK®) and *in vivo* GI of rice using Bland Altmann method (*upper limit: -6.51 and lower limit: -4.41*).

CONCLUSION

The ARK® was used to study the digestive behavior and GI of rice varieties with varying physicochemical properties. Higher cooking time, water absorption ratio, hardness, and lower solid loss were observed in Rv3 due to the presence of the compact outer bran layer. Upon digestion in the ARK®, the pH profile was higher for Rv3, with lesser SGF absorption and particle breakdown. Further, Rv2 and Rv3 had higher $t_{1/2}$ than Rv1. Also, the GI of Rv2 and Rv3 was lesser; higher amylose content (Rv2) and the presence of the fiber-rich bran layer (Rv3) retarded the starch hydrolysis. During digestion in the ARK®, levels of particle breakdown and diffusion of SGF into rice were found to be much higher than in the shaker. In terms of morphology, stomach capacity, gastric secretion flow rate, pH, and $t_{1/2}$, the ARK® reproduced *in vivo* observations. Also, the contraction action of the pneumatic piston arrangements could generate mechanical forces during gastric digestion, resulting in effective particle breakdown. Using particle size and pH differences, the predictive *in silico* algorithm successfully predicted the glucose responses for the rice varieties. In the future, this model could be used to predict glycemic responses for the specified varieties of rice without a human blood sampling procedure. Thus, the results of this study confirm that theARK® could be

used as an improved alternative for *in vitro* digestion studies. Future studies can elaborate on the digestion behavior of complex mixed meals.

Acknowledgment: Financial support for this research was given by the Department of Science and Technology (DST), Government of India, under the INSPIRE Ph.D. fellowship (Grant No: IF180587).

REFERENCES

AOAC. (2016). Official methods of analysis of AOAC International. Rockville, MD: AOAC International, ISBN: 978-0-935584-87-5.

Arvidsson-lenner, R., Asp, N., Axelsen, M., Bryngelsson, S., Haapa, E., Ja, A., … Vessby, B. (2004). Glycaemic Index. *Journal of Nutrition*, *1981*(2), 84–94. https://doi.org/10.1080/11026480410033999.

Bornhorst, G. M., Chang, L. Q., Rutherfurd, S. M., Moughan, P. J., & Singh, R. P. (2013). Gastric emptying rate and chyme characteristics for cooked brown and white rice meals in vivo. *Journal of the Science of Food and Agriculture*, *93*(12), 2900-2908. https://doi.org/10.1002/jsfa.6160.

Bornhorst, G. M., Kostlan, K., & Singh, R. P. (2013). Particle Size Distribution of Brown and White Rice during Gastric Digestion Measured by Image Analysis. *Journal of Food Science*, *78*(9), E138-91. https://doi.org/10.1111/1750-3841.12228.

Chang, U. J., Hong, Y. H., Jung, E. Y., & Suh, H. J. (2014). Rice and the Glycemic Index, 357–363. https://doi.org/10.1016/B978-0-12-401716-0.00027-1.

Ferrua, M. J., Kong, F., & Singh, R. P. (2011). Computational modeling of gastric digestion and the role of food material properties. *Trends in Food Science & Technology*, *22*(9), 480-491. https://doi.org/10.1016/j.tifs.2011.04.00.

Gopirajah, R., & Anandharamakrishnan, C. (2016). Advancement of imaging and modeling techniques for understanding gastric physical forces on food. *Food Engineering Reviews*, *8*(3), 323-335. https://doi.org/10.1007/s12393-016-9140–9148.

Gopirajah, R., Raichurkar, K. P., Wadhwa, R., & Anandharamakrishnan, C. (2016). The glycemic response to fibre rich foods and their relationship with gastric emptying and motor functions: an MRI study. *Food & Function*, *7*(9), 3964-3972. https://doi.org/10.1039/c6fo00659k.

Hasjim, J., Ai, Y., & Jane, J. (2013). Resistant Starch: Sources, Applications and Health Benefits. In *Resistant starch sources, applications and health benefits*, (pp. 79– 94.). United Kingdom: John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118528723.

Horigane, A. K., Takahashi, H., Maruyama, S., Ohtsubo, K., & Yoshida, M. (2006). Water penetration into rice grains during soaking observed by gradient echo magnetic resonance imaging. *Journal of Cereal Science*, *44*(3), 307-316. https://doi.org/10.1016/j.jcs.2006.07.014.

International Standard Organization. (2010). *Food Products-Determination of the Glycaemic Index (GI) and Recommendation for Food Classification*.

Kim, Y., Oh, I. K., Kim, H., & Lee, S. (2019). Artificial saliva-induced structural breakdown of rice flour gels under simulated chewing conditions. *Food Science and Biotechnology*, *28*(2), 387–393. https://doi.org/10.1007/s10068-018-0468-0.

Kong, F, & Singh, R. P. (2008a). A model stomach system to investigate disintegration kinetics of solid foods during gastric digestion. *Journal of Food Science*, *73*(5), E202--E210. https://doi.org/10.1111/j.1750-3841.20.

Kong, F, & Singh, R. P. (2008b). Disintegration of solid foods in human stomach. *Journal of Food Science*, *73*(5), R67--R80. https://doi.org/10.1111/j.1750-3841.2008.

Kong, Fanbin, Oztop, M. H., Singh, R. P., & McCarthy, M. J. (2011). Physical changes in white and brown rice during simulated gastric digestion. *Journal of Food Science*, *76*(6), E450--E457. https://doi.org/10.1111/j.1750-3841.20.

Li, C., Yu, W., Wu, P., & Chen, X. D. (2020). Current in vitro digestion systems for understanding food digestion in human upper gastrointestinal tract. *Trends in Food Science & Technology*, *96*, 114–126. https://doi.org/10.1016/j.tifs.2019.12.015.

Liu, W., Fu, D., Zhang, X., Chai, J., Tian, S., & Han, J. (2019). Development and validation of a new artificial gastric digestive system. *Food Research International*, *122*(August 2018), 183–190. https://doi.org/10.1016/j.foodres.2019.04.015.

Mackie, A. R., Bajka, B. H., Rigby, N. M., Wilde, P. J., Alves-Pereira, F., Mosleth, E. F., … Salt, L. J. (2017). Oatmeal particle size alters glycemic index but not as a function of gastric emptying rate. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *313*(3), G239--G246. https://doi.org/10.1152/ajpgi.00005.20.

Malagelada, J.-R., Go, V. L. W., & Summerskill, W. H. J. (1979). Different gastric, pancreatic, and biliary responses to solid-liquid or homogenized meals. *Digestive Diseases and Sciences*, *24*(2), 101-110. https://doi.org/10.1007/BF01324736.

Mano, F., Ikeda, K., Joo, E., Yamane, S., Harada, N., & Inagaki, N. (2018). Effects of three major amino acids found in Japanese broth on glucose metabolism and gastric emptying. *Nutrition*, *46*, 153-158. https://doi.org/10.1016/j.nut.2017.08.007.

Mennah-govela, Y. A., Bornhorst, G. M., & Singh, R. P. (2015). Acid Diffusion into Rice Boluses is Influenced by Rice Type , Variety , and Presence of α - Amylase, *80*(2), 316–325. https://doi.org/10.1111/1750-3841.12750.

Mohan, V., Ruchi, V., Gayathri, R., Bai, M. R., Shobana, S., Anjana, R. M., … Sudha, V. (2017). Hurdles in brown rice consumption. In *Brown rice* (pp. 255-269). Springer. https://doi.org/10.1007/978-3-319-59011–0.

Pletsch, E. A., & Hamaker, B. R. (2018). Brown rice compared to white rice slows gastric emptying in humans. *European Journal of Clinical Nutrition*, *72*(3), 367–373. https://doi.org/10.1038/s41430-017-0003-z

Priyadarshini, S. R., Arunkumar, E., Moses, J. A., & Anandharamakrishnan, C. (2021). Predicting human glucose response curve using an engineered small intestine system in combination with mathematical modeling. *Journal of Food Engineering*, *293*, 110395. https://doi.org/10.1016/j.jfoodeng.2020.11.

Ranganathan, S., Vasikaran, E. M., Elumalai, A., Moses, J. A., & Anandharamakrishnan, C. (2021). Gastric emptying pattern and disintegration kinetics of cooked rice in a 3D printed in vitro dynamic digestion model ARK®. *International Journal of Food Engineering*, *17*(5), 385-393. https://doi.org/10.1515/ijfe-2020–0159.

Ruchi, V., Mohan, V., Ramya Bai, M., & Sudha, V. (2014). Glycemic index of Indian cereal staple foods and their relationship to diabetes and metabolic syndrome. *Wheat and Rice in Disease Prevention and Health*, 333-346. https://doi.org/10.1016/B978-0-12-401716.

Santhi Rajkumar, P., Suriyamoorthy, P., Moses, J. A., & Anandharamakrishnan, C. (2020). Mass transfer approach to in-vitro glycemic index of different biscuit compositions. *Journal of Food Process Engineering*, e13559. https://do.org/10.1111/jfpe.13559.

Sethupathy, P., Moses, J. A., & Anandharamakrishnan, C. (2020). Food Oral Processing and Tribology: Instrumental Approaches and Emerging Applications. *Food Reviews International*, 1–34. https://doi.org/10.1080/87559129.2019.1710749.

Sethupathy, P., Sivakamasundari, S. K., Moses, J. A., & Anandharamakrishnan, C. (2020). Effect of varietal differences on the oral processing behavior and bolus properties of cooked rice. *International Journal of Food Engineering*. https://doi.org/10.1515/ijfe-2020-0097.

Shobana, S., Jayanthan, M., Sudha, V., Unnikrishnan, R., Anjana, R. M., & Mohan, V. (2017). Glycaemic properties of brown rice. In *Brown Rice* (pp. 123-133). Springer. https://doi.org/10.1007/978-3-319-59011–0.

Sivakamasundari, S. K., Moses, J. A., & Anandharamakrishnan, C. (2020). Effect of parboiling methods on the physicochemical characteristics and glycemic index of rice varieties. *Journal of Food Measurement and Characterization*, *14*(4), 1-16. https://doi.org/10.1007/s11694-020-00551–00559.

Sivakamasundari, S. K., Priyanga, S., Moses, J. A., & Anandharamakrishnan, C. (2020). Impact of processing techniques on the glycemic index of rice. *Critical Reviews in Food Science and Nutrition*, 1–22. https://doi.org/10.1080/10408398.2020.18652.

Somaratne, G. M., Prasantha, B. D. R., Dunuwila, G. R., Chandrasekara, A., Wijesinghe, D., & Gunasekara, D. C. S. (2017). Effect of polishing on glycemic index and antioxidant properties of red and white basmati rice. *Food Chemistry*, *237*, 716-723. https://doi.org/10.1016/j.foodchem.2017.0.

Stacher, G., Bauer, P., Schulze, D., Pointner, H., & Landgraf, M. (1976). Effect of alterations of blood glucose levels on gastric acid secretion, plasma gastrin, and plasma osmolality in man. *The American Journal of Digestive Diseases*, *21*(7), 563-568. https://doi.org/10.1007/BF01464764.

Syahariza, Z. A., Sar, S., Hasjim, J., Tizzotti, M. J., & Gilbert, R. G. (2013). The importance of amylose and amylopectin fine structures for starch digestibility in cooked rice grains. *Food Chemistry*, *136*(2), 742-749. https://doi.org/10.1016/j.foodchem.2012.0.

Van Wey, A. S., Cookson, A. L., Roy, N. C., McNabb, W. C., Soboleva, T. K., Wieliczko, R. J., & Shorten, P. R. (2014). A mathematical model of the effect of pH and food matrix composition on fluid transport into foods: An application in gastric digestion and cheese brining. *Food Research International*, *57*, 34-43. https://doi.org/10.1016/j.foodres.2014.01.0.

Wang, J., Wu, P., Liu, M., Liao, Z., Wang, Y., Dong, Z., & Chen, X. D. (2019). An advanced near real dynamic in vitro human stomach system to study gastric digestion and emptying of beef stew and cooked rice. *Food & Function*, *10*(5), 2914-2925. https://doi.org/10.1039/c8fo02586j.

Ye, J., Liu, C., Luo, S., Wu, J., Hu, X., & McClements, D. J. (2019). A simulated gastrointestinal tract study of texturized rice grains: Impact of texturization on starch digestibility. *Journal of Cereal Science*, *89*, 102800. https://doi.org/10.1016/j.jcs.2019.102800.