Original paper

Development of a Human Gastric Digestion Simulator Equipped with Peristalsis Function for the Direct Observation and Analysis of the Food Digestion Process

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A novel *in vitro* gastric device, the Gastric Digestion Simulator (GDS), was developed for the direct observation and quantitative analysis of the food digestion process in the human stomach. In addition to simulating the chemical digestion environment, this device provides a physical digestion environment comparable to that found in the stomach by simulating peristalsis, which is assumed to contribute to solid food disintegration. The GDS was successfully used to directly observe the disintegration process of *Tofu* (bean curd) as a typical solid food containing protein. The size distribution and protein content of *Tofu* particles during the digestion experiments were investigated. The results demonstrated the difference in particle disintegration between GDS and flask shaking experiments, which may be due to the lack of peristalsis in the latter case. Moreover, the size distribution of *Tofu* particles after the GDS experiments was affected by the physical properties of *Tofu*, thus revealing the usefulness of GDS for food digestion analysis.

Keywords: GI tract, gastric digestion, *in vitro* gastric device, peristalsis, direct observation, solid food

Introduction

In the human gastrointestinal (GI) tract, foods are digested by a combination of physical and chemical processes. Chemical digestive processes are catalyzed by digestive enzymes secreted in the stomach and small intestine, and disintegrate foods down to the molecular scale. However, physical digestive processes, which are induced mainly by peristalsis, mix and empty gastric and intestinal contents, and thus play an important role in promoting food digestion in the GI tract. Peristalsis in the GI tract is caused by peristaltic wall motion (*e.g.*, antral contraction waves (ACWs)) in the stomach. Periodically generated ACWs induce the motion of the gastric contents, mix the gastric contents, and grind bulk solid foods to reduce their particle size (Kong and Singh, 2008). Several research groups have reported that fluid motions in the stomach

promote the emulsification of oil components and drug release from matrix tablets (Schwizer *et al.*, 2006; Abrahamsson *et al.*, 2005). Ajaj *et al.* (2004) reported unusual peristaltic motions in gastroparesis patients having a decreased ACW speed or different degrees of ACW contractions, which were monitored by magnetic resonance imaging (MRI). However, the physical effects of gastric peristalsis on gastric digestion are not yet fully understood. Such investigation may provide useful information for designing new foods with precisely controlled digestion properties.

Food digestion in the human GI tract has been studied using *in vivo*, *in vitro*, and *in silico* approaches. MRI has been used for *in vivo* studies on gastric peristaltic motion and the physical properties of gastric contents. The speed of ACWs and the ACW amplitude in the human stomach were calculated using data obtained by real-

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time MRI (King *et al.*, 1984; Pallotta *et al.*, 1998). The time-dependent change of the viscosity of the gastric contents was measured using echo planar MRI (Marciani *et al.*, 2000). Marciani *et al.* (2001) also visualized the concentration distribution of test liquid meals in the human stomach. Although these findings provide physical insights into gastric peristalsis and intragastric fluid motion, the observation or quantitative analysis of complex food digestion processes (*e.g.*, solid food disintegration) would be difficult.

Shaking-flask and test-tube methods are conventionally used for evaluating *in vitro* GI digestion (McClements *et al.*, 2010; Kong *et al.*, 2008). These methods can simulate the chemical environment in the GI tract (*e.g.*, pH, salt, and digestive enzymes) inside a test tube. Van Aken *et al.* (2011) reported *in vitro* gastric digestion of oil-in-water emulsions that dynamically simulated the chemical environment in a glass stirring vessel equipped with a port for adding gastric fluid. However, it is difficult to use these *in vitro* digestion methods to simulate the dynamic physical environment of the GI tract, such as mixing and emptying the GI contents by peristaltic motion.

Several dynamic gastric or gastrointestinal devices have been proposed in the last two decades. A dynamic GI tract device was developed at The Netherlands Organization for Applied Science Research (TNO). Their model, the TIM, can automatically expand and contract the flexible tubular walls in the simulated GI tract and control the pH, temperature, and the secretion of GI fluids (Minekus *et al.*, 1995; Blanquet-Diot *et al.*, 2004; Blanquet-Diot *et al.*, 2009). The dynamic gastric model (DGM) developed at the Institute of Food Research (IFR) is a fully automated human gastric system that allows secretion of gastric fluid and gastric emptying using a fixed outer cylinder and a movable inner cylinder (Mercuri *et al.*, 2008). These devices are useful for dynamically simulating the chemical digestive processes of foods, but not for bulk solid foods that require disintegration by physical digestion. The peristaltic motion modeled in TIM and DGM does not incorporate ACW, which plays a major role in physical digestion. Therefore, several gastric devices that focus mainly on physical digestion have recently been developed. A simple gastric device proposed by Chen *et al.* (2011) can simulate gastric mixing flow by upward and downward motions of a spherical Teflon probe. Kong and Singh (2010) developed a Human Gastric Simulator (HGS) that can generate a gastric peristalsis-like progressing wave on a gastric wall made of opaque latex and a roller rotation system. However, directly monitoring the digestion and size distribution of the gastric contents using HGS is difficult.

Dynamic intragastric flow phenomena have been visualized mainly by *in silico* approaches using the lattice Boltzmann and computational fluid dynamics (CFD) methods. Several research groups have reported the visualization of flow phenomena induced by human gastric peristalsis (Pal *et al.*, 2004; Ferrua *et al.*, 2010; Kozu *et al.*, 2010; Xue *et al.*, 2012). Pal *et al.* (2004) predicted two

representative gastric fluid motions: retropulsive jet flow through ACW, and circulatory flow behind ACW. We recently analyzed the effect of the viscosity of liquid gastric contents on the gastric flow-field (Kozu *et al.*, 2010). These *in silico* studies simulated only the one phase fluid condition because it is difficult to simulate the motion of multiphase gastric contents.

This study seeks to develop a new *in vitro* gastric digestion device that focuses mainly on the physical digestion environment of the human stomach, and to assess the device's performance. The gastric digestion simulator (GDS) that we developed here can simulate peristaltic motion in the human stomach and enables direct observation of the digestion process in real time. In this study, two different types of *Tofu* were used as model solid foods. We investigated food digestion characteristics using GDS to quantitatively understand the dynamic digestion processes in the human stomach.

Materials and Methods

Development of GDS The GDS (Fig. 1) was developed to quantitatively analyze and visualize the disintegration of food particles in the human stomach. The GDS consists of a gastric vessel, a roller system for inducing peristaltic motion, a temperature control system, and a transparent plastic chamber. The gastric vessel that simplifies the distal part of the human stomach, known

Fig. 1. Photograph of Gastric Digestion Simulator (GDS) including the gastric vessel, rollers, and temperature control systems.

as the antrum, has parallel transparent walls on one plane and rubber sidewalls on another plane. This gastric vessel enables direct observation of gastric digestion behavior through the transparent walls. The sidewalls are deformable to allow the generation of progressing waves that model ACWs. The device is equipped with a temperature control system consisting of a ribbon heater and a temperature sensor.

The simplified geometry and dimensions of the gastric vessel are presented in Fig. 2(a). This gastric vessel was designed to simulate the antrum, where peristalsis induces motion of the gastric contents. The transparent planes of the vessel have a trapezoidal shape, as the human antrum has a tapered structure, with a smaller inner diameter toward the pylorus (Pal *et al.*, 2004). The vessel has a total volume of 550 mL; its dimensions are presented in Fig. 2(a).

As depicted in Fig. 2(b), progressing waves that model ACWs can be generated on the sidewalls of the gastric vessel by pushing a

pair of rollers onto the deformable walls. Each side of the roller system consists of three polyethylene rollers with a diameter of 45 mm, two timing belts, and a motor. The rollers are turned inward, and move down at a constant speed and ACW generation frequency along the vessel's sidewalls. The rollers' moving track image is denoted as dashed arrows in Fig. 2(b). The rollers progress at several millimeters per second with a frequency of a few cycles/min, corresponding to human ACW parameters (Schwizer *et al.*, 2006). As the rollers progress downward along the sidewalls, the clearance (distance between rollers) gradually decreases, reaching a minimum of 5.4 mm. This value is comparable to *in vivo* data (Pal *et al.*, 2004).

Material preparation and properties Tofu (bean curd) was used in this study as a model protein-based food. Tofu is generally used in food studies as a solid food that contains 85 to 90% water and 5 to 10% protein, according to the Standard Tables of Food

Fig. 2. Simple geometry of GDS. (a) 3-D schematic of the gastric vessel. (b) Schematic of the roller systems. Oval dashed lines in (b) denote the tracks of roller rotation.

Table 1. Physical properties of *Tofu* samples prior to digestion. Significant differences for breaking stress (* $p < 0.01$) and Young's modulus (** $p < 0.05$) are indicated

<i>Tofu</i>	Breaking stress (kPa)	Young's modulus (kPa)
<i>Kinugoshi</i>	11.5 ± 0.7 *	24.4 ± 3.1 **
<i>Momen</i>	20.1 ± 2.9 *	28.9 ± 2.8 **

Composition in Japan (i). Two types of *Tofu*, *Kinugoshi* and *Momen*, were purchased at a local market. Although the chemical composition of these two types is nearly the same, their manufacturing processes differ. *Kinugoshi* is made from soy milk gel without draining the water, whereas *Momen* is made from the same raw materials but the water is drained afterward. Therefore, their physical properties are generally different.

For digestion fluid preparation, NaCl, KCl, NaHCO₃, and HCl were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). For the digestion enzymes, α -amylase (#02100447) was purchased from MP Biomedicals, Inc. (Santa Ana, USA), and pepsin from porcine gastric mucosa P7000 was purchased from Sigma-Aldrich, Inc. (St. Louis, USA).

The breaking stress and Young's modulus of *Kinugoshi-Tofu* and *Momen-Tofu* were measured before the digestion experiment. Texture profile analysis was applied using a TPU texture profile unit (Yamaden Co., Ltd., Tokyo, Japan). An 8.0 mm-diameter probe was used in the texture measurements. Samples cubes (15 mm x 15 mm x 15 mm) were compressed and deformed up to 90% using a probe speed of 1.0 mm/s. Their breaking stress and Young's modulus are presented in Table 1.

In vitro gastric digestion procedures

(1) Preparation of simulated saliva and gastric juice

Simulated saliva was prepared by dissolving 0.117 g/L NaCl, 0.14 g/L KCl, 2.1 g/L NaHCO₃, and 2.0 g/L α -amylase in Milli-Q water. Simulated gastric juice was prepared by dissolving 8.775 g/L NaCl and 1.0 g/L pepsin in Milli-Q water and adjusting to pH 1.3 using 1 N HCl.

(2) Preparation of *Tofu* as a model food for digestion simulation

Considering that foods are chewed before digestion, each *Tofu* sample was cut into 5.0 mm cubes before the gastric digestion experiment; 80 g of *Tofu* particles were then gently mixed with 30 mL of the simulated saliva in a beaker and allowed to stand for 2 min. Next, 200 mL of the simulated gastric juice was added to the *Tofu*-saliva mixture. All simulated gastric contents were prepared at 37°C.

(3) GDS experiment

The GDS developed in this study was used to demonstrate physical effects on solid food digestion, as well as enabling visualization of the digestion process. Simulated gastric contents (each containing 80 g *Tofu*, 30 mL saliva, and 200 mL gastric juice,

previously prepared) were transferred to the GDS and incubated at a constant temperature of 37°C for up to 180 min. The applied ACW speed was 2.5 mm/s, and the generation frequency was 1.5 cycles/min. The entire digestion process in the GDS was monitored using a video camera (Fig. 2(a)), and the time course changes of the *Tofu* particles were monitored by analyzing their packing, shape, and size.

(4) Flask-shaking experiment

To compare the GDS and conventional *in vitro* gastric digestion experiments, a flask-shaking experiment was conducted according to the procedure described by Wang *et al.* (2013) with a slight modification. In this experiment, simulated gastric contents were transferred to a 300 mL Erlenmeyer flask and incubated at 37°C using a shaking frequency of 115 strokes/min, for up to 180 min.

Characterization of digested *Tofu* samples

(1) Classification and observation

The digesta containing *Tofu* particles after the GDS or flask-shaking experiment were classified using sieves of four different mesh sizes: 0.60, 1.18, 2.36, and 3.35 mm. After sieving, photographs of the *Tofu* particles collected in each fraction were taken, and their particle shape was observed.

(2) Dry weight measurement

To determine the *Tofu* particle size distribution after digestion, the dry weight of each size fraction was measured. *Tofu* particles collected from the four size fractions, plus the smallest fraction ($d < 0.60$ mm) containing digesta with fine *Tofu* particles, were dried to constant weight in a vacuum oven (AVO-250NS, As One Co., Ltd, Osaka, Japan) and the dry weight of each size fraction was measured.

(3) Crude protein analysis

To determine the protein content of the sieved *Tofu* particles, crude protein was analyzed using a combustion test (2400II CHN Analyzer, PerkinElmer, Inc., Waltham, USA). Three milligrams of dried sample from each size fraction were burned and converted into gas containing CO₂, H₂O, and NO_x. The gases were separated through a column according to the principle of frontal chromatography (Uhdeova and Rezl, 1981). The nitrogen content of each burned sample was measured using a CHN Analyzer sensor. Since the average nitrogen content of proteins is 16%, the crude protein content in each sample was estimated by multiplying the measured nitrogen weight by a conversion factor of 6.25 (Furuta *et al.*, 1998).

Statistical analysis Analysis of variance (ANOVA) was conducted to evaluate significant differences in the breaking stress and Young's modulus between *Kinugoshi-Tofu* and *Momen-Tofu*. The maximum confidence level was 95% ($p < 0.05$). All measurements of physical properties were repeated six times ($n = 6$).

Results and Discussion

ACWs simulated in GDS Figure 3 presents snapshots of the

movement of ACWs simulated on the sidewalls of the gastric vessel filled with simulated gastric juice. A pair of contraction waves on the sidewalls was generated periodically by turning the rollers inward. The ACWs generated at an interval of 40 s progressed towards the base of the gastric vessel. The clearance between the deformed sidewalls narrowed to 5.5 mm as the ACWs progressed downward.

The simulated ACWs compress and mix the gastric contents inside the vessel. Previous simulation studies analyzing the mixing flow-field induced by ACWs based on the *in silico* approach demonstrated flow opposing the progressing direction of ACWs and a maximum flow velocity of approximately 10 mm/s (Pal *et al.*, 2004; Kozu *et al.*, 2010). Such mixing gastric flow behavior has been also demonstrated using the *in vitro* approach with simplified gastric devices (Ferrua *et al.*, 2010; Kobayashi *et al.*, 2013). Those authors reported that ACWs were simulated by soft wall deformation. These studies suggest that ACWs generated by GDS have similar flow characteristics.

Direct observation of the digestion process in GDS Figure 4

presents snapshots of *Kinugoshi-Tofu* digestion in GDS up to 180 min. Initial *Tofu* particles (5.0 mm cubes) gradually disintegrated into smaller pieces, thus reducing the total size of the particles, which were packed more densely. The same tendency was observed in digestion experiments using *Momen-Tofu*. The appearance of the liquid phase in the gastric contents also changed during the digestion experiments, regardless of the *Tofu* type used. At first, the digestive fluid above the packed *Tofu* particles was almost transparent; however, it gradually became turbid. Moreover, fine *Tofu* particles were dispersed in the digestive fluid throughout the experiment.

Although the size reduction of food particles after a digestion experiment was previously reported using an *in vitro* gastric device (Kong and Singh, 2010), the disintegration mechanism was not well understood. Thus, the digestion process observed in GDS provides useful insights into food disintegration.

Analysis of dry weight of each particle size fraction

(1) Kinugoshi-Tofu using GDS

Figure 5(a) presents the variation in the dry weight of each size

Fig. 3. Snapshots of ACW generated in one cycle. The gastric vessel was filled with simulated gastric juice. White dashed circles indicate the momentary position of the rollers.

Fig. 4. Snapshots of *Kinugoshi-Tofu* digestion up to 180 min. Disintegration of *Tofu* particles was directly observed.

fraction of *Kinugoshi-Tofu* particles during the GDS digestion experiments. The largest fraction ($d > 3.35$ mm), including the initial 5 mm *Tofu* particles, decreased rapidly up to 17% for 120 min digestion, reaching 12% after 180 min. However, the weight ratio of the intermediate fraction (0.60 mm $< d < 3.35$ mm) gradually increased to 41% at 120 min and reached 43% within 180 min. The smallest fraction ($d < 0.60$ mm) increased to 30% at 120 min and reached 40% at 180 min. These data indicated that *Tofu* particles with an initial size of 5.0 mm gradually disintegrated and shifted toward small size fractions, corroborating the direct observation results presented in Fig. 4.

Kinugoshi-Tofu particles in each size fraction after 180 min digestion are depicted in Fig. 6(a). Although the initial *Tofu* particles were cubic, the particles that disintegrated during digestion were irregularly shaped, suggesting that they were compressed and broken down by sidewall contractions in the gastric vessel. The particles were smaller than the minimum sidewall clearance, and the loosened contact between particles led to ineffective disintegration. However, when particles were densely compacted, they broke down more easily by crushing against each other, which may also occur in the human stomach. In addition, *Tofu* particles can be broken down by being squeezed between the

Fig. 5. Dry weight in each size fraction of *Tofu* particles during digestion experiments, indicating time change of size distribution. Digestion of *Kinugoshi-Tofu* by (a) GDS and (b) flask shaking. (c) Digestion of *Momen-Tofu* by GDS. The error bar at 0 min stands for the standard deviation of dry weight ($n = 5$).

Fig. 6. Typical shape of disintegrated *Kinugoshi-Tofu* particles in each fraction after 180 min digestion using (a) GDS and (b) flask shaking.

end of the GDS simulated pylorus and peristalsis progressing toward the end of the GDS. This type of compression process would probably occur in the human stomach. In some cases, the gastric contents in the actual stomach are more viscous, depending on the type, amount, and composition of foods (Marciani *et al.*, 2000). Increased viscosity of the gastric fluid is assumed to promote food breakdown since a high hydrodynamic pressure would impose pressure on the gastric contents for longer.

(2) *Kinugoshi-Tofu* using flask-shaking digestion

Figure 5(b) presents the variation in the dry weight of each size fraction of *Kinugoshi-Tofu* particles during flask-shaking digestion. The largest fraction decreased almost linearly to 37% after 180 min digestion. The ratio of the intermediate fraction increased smoothly up to 10% within the same period. The smallest fraction also gradually increased, reaching 55% at the end of the digestion experiment. The ratio of the intermediate fraction during digestion differed between the GDS and flask-shaking experiments. As presented in Fig. 6(b), the largest and intermediate fractions of *Tofu* particles maintained their cubic shape with smooth edges, even after digestion. These results indicate that, for the flask-

shaking system used, the surfaces of the *Tofu* particles were dissolved by the chemical effect of the digestion juice, reducing their size without crushing.

(3) *Momen-Tofu* in GDS

Figure 5(c) presents the variation in the dry weight of each size fraction of *Momen-Tofu* particles during the GDS digestion experiments. The largest fraction gradually decreased to 13% after 180 min digestion. The ratio of the intermediate fraction increased to 30% within the same period, whereas the smallest fraction increased more, to 58% at 180 min.

Although the disintegration tendency was similar for both the *Kinugoshi-Tofu* and *Momen-Tofu* particles, the variation of the ratio of each size fraction was influenced by the *Tofu* type. For instance, *Kinugoshi-Tofu* particles in the largest fraction disintegrated more easily and quickly than *Momen-Tofu* particles. These results could be due to the different breaking stress and Young's modulus: the breaking stress and Young's modulus of *Momen-Tofu* were significantly higher than those of *Kinugoshi-Tofu* (Table 1).

Designing the physical properties of foods is important, especially for solid food digestion. The GDS digestion results using

Fig. 7. Time changes of crude protein in each fraction during GDS experiments. (a) *Kinugoshi-Tofu*. (b) *Momen-Tofu*. The error bar at 0 min stands for the standard deviation of crude protein ($n = 3$).

Kinugoshi-Tofu and *Momen-Tofu* can provide insights for developing novel foods with precisely controlled digestibility.

Crude protein analysis at different GDS digestion periods The amount of crude protein was analyzed in each size fraction after the GDS digestion experiments using *Kinugoshi-Tofu* (Fig. 7(a)) and *Momen-Tofu* (Fig. 7(b)) to quantitatively investigate the change in the protein content of each *Tofu*. The variations in the three major fractions were similar for both crude protein amount and dry weight (Figs. 5(a) and (c)), which may be associated with the nearly constant nitrogen concentration (7 to 10%) in each size fraction. Figure 8 presents the crude protein ratio of the size fraction smaller than 2.36 mm based on Figs. 7(a) and (b). This mesh size is close to the pylorus diameter in the human stomach, where gastric emptying takes place. The crude protein ratio of the fraction smaller than 2.36 mm increased continuously with digestion time for both *Kinugoshi-Tofu* and *Momen-Tofu*. After 180 min digestion, the crude protein ratio exceeded 70%, which corresponds to their dry weight ratio. These results demonstrated that *Tofu* protein was contained mostly in the fraction smaller than the pylorus

Fig. 8. Changes in the ratio of crude protein in fractions smaller than 2.36 mm compared to all size fractions during digestion.

diameter after GDS digestion, which indicates that this size of digested *Tofu* particles could be transported into the small intestine.

Conclusions

GDS was developed as a new *in vitro* gastric digestion device to simulate the environment in the human stomach. The device focuses mainly on physical digestion effects by peristalsis. This device enabled the direct observation of food disintegration, as well as changes in the gastric contents, in real time. These observations are difficult using other recently-described GI-tract devices. Also, we quantitatively analyzed changes in the size distribution and protein content of *Tofu* particles during digestion using GDS. The size distribution after the GDS digestion experiments differed from that obtained by flask-shaking experiments, and it also changed with different physical properties of *Tofu*. The use of GDS demonstrated the disintegration of solid foods by both physical and chemical digestion effects. Our findings are expected to help understand food digestion processes in the human stomach and to contribute to the design of foods for controlled digestion.

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