Original paper

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

Hiroyuki Kozu¹², Yuki Nakata², Mitsutoshi Nakajima¹², Marcos A. Neves¹², Kunihiko Uemura¹, Seigo Sato², Isao Kobayashi¹* and Sosaku Ichikawa²*

¹Food Engineering Division, National Food Research Institute, NARO, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan
²Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8572, Japan

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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-
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 tempera-
ture 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.](image)
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.](image-url)
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.

<table>
<thead>
<tr>
<th>Tofu</th>
<th>Breaking stress (kPa)</th>
<th>Young’s modulus (kPa)</th>
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<tbody>
<tr>
<td>Kinugoshi</td>
<td>11.5±0.7 *</td>
<td>24.4±3.1 **</td>
</tr>
<tr>
<td>Momen</td>
<td>20.1±2.9 *</td>
<td>28.9±2.8 **</td>
</tr>
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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 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. 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 digests 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 NOₓ. The gases were separated through a column according to the principle of frontal chromatography (Uhrdeova 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.](image-url)
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 \(d > 3.35 \text{ mm}\), 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 \((0.60 \text{ mm} < d < 3.35 \text{ mm})\) gradually increased to 41% at 120 min and reached 43% within 180 min. The smallest fraction \(d < 0.60 \text{ mm}\) 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 wall 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 (\(n = 5\)).
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
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 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 contribute to the design of foods for controlled digestion.

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References


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