Analysis of Disintegration of Agar Gel Particles with Different Textures using Gastric Digestion Simulator

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This study investigates the effect of the texture of agar gel as a model solid food on gastric digestion using a human Gastric Digestion Simulator (GDS). The GDS, which simulates gastric peristalsis, can investigate physical digestion phenomena such as disintegration of solid foods. Agar gels with different fracture stresses (56 to 219 kPa) and constant fracture strain (29%) were prepared by varying the agar concentration (1.5 to 4.5 wt%). Direct observation demonstrated that agar gel particles initially cut to a 5.0 mm cube gradually reduced in size and broke down into random shapes during simulated gastric peristalsis. The size distribution of the agar gel particles after the digestion experiment was analyzed using the sieving method. The fraction larger than 2.36 mm, which corresponds to the pylorus size, decreased with time: the wet weight ratio of that fraction to the total amount of agar gel particles was 18.0% at 180 min in the case of 1.5 wt% agar gel. The agar gel concentration did not affect the size distribution after 180 min, which shows that fracture strain plays a more important role in agar gel digestion. Our results provide useful information about the relationship between solid food texture and gastric digestion.

Keywords: Gastric digestion, peristalsis, in vitro, Agar gel, Food texture

1. Introduction

The human stomach is an important organ in food digestion. Both physical and chemical digestive effects play important roles in gastric digestion. Gastric juice, which contains HCl and digestive enzymes, catalyzes chemical reactions of disintegrating foods down to a molecular scale. In addition, physical digestive processes are promoted by gastric peristalsis, which involves the peristaltic motion of the gastric wall called the Antral Contraction Wave (ACW) [1]. Gastric peristalsis is assumed to have the functions of mixing the gastric contents (a mixture of food and digestive juice), breaking down foods, and emptying the gastric contents into the intestine [2]. It is important to study these digestion phenomena in the stomach, since such information is useful to the food industry, for example in controlling the physical properties of foods to make them more easily digested.

Chemical digestion in the stomach has been studied mainly by in vitro approaches. Chemical environments including pH, salt, and digestive enzymes have been simulated using artificial digestive juices inside test vessels [3-6]. A more complex digestion system has also been proposed [7-8]. Physical digestion is especially important in the case of solid food, since the size reduction of solid food by breaking down must promote enzyme reactions. Also, disintegration of solid food structures can contribute to the release of nutrients inside the solid food. Marciani et al. investigated the breaking down of solid food in the human stomach in vivo [9]. Physical digestion of solid foods has also been studied in vitro by placing a mechanical load representing solid foods and using a piston and barrel movement [10] or simulated peristalsis movement [11]. These in vivo and in vitro studies indicate that the physical properties of solid food can affect disintegration in the stomach. However, physical digestion of solid foods in the stomach is not sufficiently understood. For instance, the dominant texture parameter affecting the disintegration of solid food is not clear.

We have developed an in vitro device called the Gastric Digestion Simulator (GDS) to investigate chemical and physical effects on food digestion in the human stomach.
Gastric peristalsis, a capability which is mainly used to analyze the digestion of solid foods. In our previous study, Tofu, which is a typical solid food, was used to analyze gastric digestion by GDS [12]. The results using two kinds of Tofu indicate that different Tofu textures may affect disintegration in the stomach. Thus, a more fundamental study focusing on the relationship between food texture and disintegration in the stomach is needed to obtain essential information about controlling solid food digestion.

This study seeks to analyze the effect of food texture on gastric digestion using GDS. Agar gel was used as a model food to investigate the physical digestion effect. Agar is a major polysaccharide that is hardly disintegrated by gastric acid and enzyme, so we can analyze pure physical digestion of samples. We prepared various concentrations of agar gel, controlling its texture, and quantitatively analyzed the effect of agar gel texture on digestion in the stomach.

2. Materials and methods

2.1 Materials

Agar powder (#010–15815) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). NaCl, KCl, NaHCO3, HCl, H2SO4, and phenol were purchased from the same company. \( \alpha \)-Amylase (#02100447) was purchased from MP Biomedicals, Inc. (Santa Ana, CA, USA), and pepsin from porcine gastric mucosa P7000 was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA) for the digestion enzymes.

2.2 Sample preparation and texture analysis

To prepare simulated saliva, 0.117 g/L NaCl, 0.14 g/L KCl, 2.1 g/L NaHCO3, and 2.0 g/L \( \alpha \)-amylase were dissolved 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. The pH of simulated gastric juice was adjusted to 1.3 using 1N HCl.

Agar gels with different concentrations (1.5 to 4.5 wt%) were used to vary the texture. Each concentration of agar powder was dissolved in Milli-Q water by heating. The agar solution was then gelatinized by cooling it to 5°C. As typical texture parameters, the fracture stress and fracture strain of the prepared agar gels were measured by the texture profile analysis method (texture profile unit (TPU), Yamaden Co., Ltd., Tokyo, Japan). Cubic agar gels of 10 mm (width) x 10 mm (depth) x 10 mm (height) were compressed up to 90% deformation using a probe. The probe diameter was set to 40 mm and speed to 1.0 mm/s. Analysis of variance (ANOVA) was conducted to evaluate significant differences in the fracture stress and fracture strain of the agar gels at each concentration. The maximum confidence level was 95% (\( P < 0.05 \)). All measurements were repeated five times (\( n = 5 \)).

2.3 In vitro gastric digestion procedures

Considering the chewing of food, 120 g of agar gel was cut into 5.0 mm cubic particles and mixed with 30 mL simulated saliva for 2 min before the in vitro gastric digestion experiment. 150 mL of simulated gastric juice was then added to this mixture. All gastric contents were prepared at 37°C. Finally, the prepared mixture was used for the digestion experiment as simulated gastric contents. Although the amount of ingested foods is not always constant in actual digestion, these amount of agar gel and simulated gastric juice in this study was used in reference to a standardized procedure of in vitro digestion experiment [13].

GDS was used for conducting the gastric digestion experiment of agar gels, as depicted in Fig. 1. GDS models distal stomach (antrum) where gastric peristalsis mainly occurs. Total volume of GDS vessel is approximately 500 mL, considering the antrum volume estimated according to the scale of human stomach [14]. Gastric peristalsis was simulated by the deformation of rubber sidewalls compressed by polyethylene rollers (Fig. 1) [12]. The speed and frequency of gastric peristalsis were set to 2.5 mm/s and 1.5 cycle/min, representing a healthy human [15]. The gastric contents prepared in the earlier section were digested in GDS at a constant temperature of 37°C for up to 180 min. The disintegration of the agar gel particles was directly observed and monitored using a video camera through the transparent acrylic walls of the GDS vessel.

An in vitro gastric digestion experiment without peristalsis was also conducted using the flask-shaking method to clarify the peristalsis effect on agar gel disintegration. The gastric contents were loaded into a 500 mL Erlenmeyer flask and incubated at 37°C, shaking in a circumferential direction at 120 rpm for up to 180 min.

2.4 Characterization of the digested samples

The size of the agar gel particles that disintegrated after the GDS or flask-shaking experiment were analyzed using the sieving method [11–12]. Digested agar gel particles were classified using four different sieves.
with mesh sizes of 0.60, 1.18, 2.36, and 3.35 mm. In the sieving operation, gel particles were mildly washed using Milli-Q water to prevent break down of the particles during the operation. The wet weight of each size fraction was then measured to investigate the size distribution. The typical shape of the digested agar gel particles in each size fraction was observed after sieving.

The fraction smaller than 0.60 mm was collected and heated to 100°C so that the agar gel particles were completely dissolved. The concentration of agar in this fraction was measured using the phenol–sulfuric acid method. The total amount of agar was estimated using the total weight of the fraction smaller than 0.60 mm and the measured concentration of agar in that fraction. The total amount of agar of that fraction was then translated into the wet weight of agar gel particles corresponding to each initial agar gel concentration (1.5–4.5 wt%).

3. Results and discussion

3.1 Direct observation of agar gel digestion using GDS

The digestion of control agar gel particles with a concentration of 1.5 wt% is presented in Fig. 2 as a series of snapshots taken at 60 min intervals. Agar gel initially cut into 5.0 mm cubic particles disintegrated into smaller pieces in the presence of simulated gastric peristalsis. Agar gel particles disintegrated gradually until 120 min; after that, the particles slowly decreased in size up to 180 min. Because of the size reduction, the agar gel particles were packed more densely, resulting in a decrease in the packing height of the particles. Such disintegration was also observed in our previous study using Tofu [12].
3.2 Analysis of size distribution of agar gel particles after digestion experiment

3.2.1 GDS

The size distribution of control agar gel particles in GDS after 30 to 180 min digestion experiments is presented in Fig. 3 (a). The wet weight of the fraction exceeding 2.36 mm, which corresponds to the size of pylorus [2], decreased with time. The weight ratio of the fraction exceeding 2.36 mm to the initial amount of agar gel particles gradually decreased to 23.2% during the initial 120 min and slowly decreased to 18.0% up to 180 min. This size reduction tendency agrees with the observation result in Fig. 2. The total wet weight of the sieved particles (d > 0.60 mm) hardly changed for the initial 90 min and subsequently decreased up to 180 min. The total weight of the fraction smaller than 0.60 mm was 33.6 g at 180 min. The total wet weight of the agar gel, including the fraction smaller than 0.60 mm, was 113 g, which is similar to the initial amount of agar gel particles.

The typical shape of the control agar gel particles after 180 min of the GDS experiment is depicted in Fig. 3 (b). In all size fractions, the initially cubic agar gel particles physically broke down into randomly shaped particles in the presence of simulated peristalsis. The gastric contents were compressed by simulated peristalsis moving down the GDS vessel, which can promote the physical break down of the agar gel particles.

3.2.2 Flask-shaking experiment

Figure 3 (c) plots the size distribution of the control agar gel particles after the flask-shaking experiment for each digestion time. The agar gel particles hardly disintegrated, resulting in unchanged wet weight of the largest fraction (d > 3.35 mm) even at 180 min in the absence of gastric peristalsis. Chemical digestion can hardly affect agar gel disintegration, since agar is not chemically decomposed by pepsin and, the digestion temperatures in this study could be too low to promote chemically hydrolysis of agar by gastric acid. When considering the physical effects, the agar gel particles were mixed with simulated gastric juice and were crashed each other during flask-shaking experiment. However, the agar gel particles maintained their initial cubic shape in 180 min. Thus, this shaking effect must not enough to physically disintegrate agar gel particles compared to

![Fig. 3 Wet weights of each size fraction of control agar gel particles (agar concentration of 1.5 wt%) during digestion experiments using (a) GDS and (c) flask-shaking. A typical shape of the digested agar gel particles after 180 min in GDS is seen in (b).]
peristalsis. The results demonstrate that physical digestion by gastric peristalsis is dominant for solid foods rich in polysaccharides.

3.3 Effect of agar gel texture on disintegration behavior in GDS

Figure 4 plots the size distribution of agar gel particles with different concentrations digested using GDS. Agar concentration did not affect the size distribution after 180 min.

Figure 5 illustrates the variation in the fracture stress and strain of agar gels as a function of agar concentration. The fracture stress linearly increases from 56 to 219 kPa within the range of 1.5 to 4.5 wt%. This shows that fracture stress hardly affects disintegration of agar gel particles in GDS. In contrast, there was no significant difference in fracture strain (average value: 29%) in all agar gel concentrations ($P>0.05$). Physical compression by gastric peristalsis is the major factor affecting gel disintegration; i.e., the elasticity of the gel could be an important factor. The low fracture strain (30%) of agar gel causes low elasticity, so agar gel can be easily fractured by even a slight compression. Thus, fracture strain could be one of the dominant texture parameters of disintegration in the stomach in the case of agar gel. Fracture stress may also be an important factor in the case of rigid foods, since there is also the possibility that the compression force in GDS may be strong enough to disintegrate all types of agar gel used in this study. These results and discussions provide us one of the hints for understanding disintegration phenomena of solid foods in human stomach.

Although the effects of agar gel fracture force on gastric disintegration have been discussed both in vivo [9] and in vitro [10], there is no investigation on the effects of its fracture strain. Guo et al. (2014) measured several texture parameters of protein gels (elastic modulus, fracture force, and fracture strain) and conducted in vitro gastric digestion experiment using these gels [16]. However, effect of each texture parameter on gastric digestion remains unclear. Thus, individually investigating fracture stress and strain in this study help us understand the dominant texture parameters affecting solid food digestion in the stomach. The results are expected to give us insight on controlling the physical properties of solid foods that enables them to be digested more or less easily.

4. Conclusions

Agar gel digestion using GDS was analyzed to clarify the effect of food texture on gastric digestion. Direct observation demonstrates that agar gel particles gradually disintegrated in the presence of simulated peristalsis during the GDS experiment but hardly disintegrated in the absence of peristalsis, suggesting the importance of gastric peristalsis in solid food digestion. The change in gel fracture stress did not affect the disintegration, indicating that fracture strain is the dominant factor in solid food digestion in the stomach.
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References