Effects of the Type of Pectin and Concentration of Citric Acid on Digestive Behavior of a Bubble-containing Gel: Evaluation Using a Human Gastric Digestion Simulator

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Recently, a new beverage was developed that forms a bubble-containing gel and expands in the stomach, so that it suppresses hunger despite being low in calories. In order to improve the hunger suppression effect of the beverage, it is important to clarify the optimum preparation conditions of the beverage so that it stretches the stomach wall to a larger extent, and for a longer time. Factors affecting the volume of a bubble-containing gel and its volume-retention capacity were investigated by in vitro digestibility evaluation using the human Gastric Digestion Simulator (GDS). It was determined that the degree of esterification of the pectin used and concentration of citric acid in the formulation affected the expansion volume of the gel immediately upon reaction with artificial gastric juice. Furthermore, the degree of amidation of the pectin used and physical properties of the gel also affected the retention capacity of the gel volume. For maximizing the expansion volume of the bubble-containing gel and its retention capacity after consumption of this beverage, it is necessary to consider the degree of esterification and amidation and the citric acid concentration in the formulation.

Keywords: Pectin, Gel, Beverage, Digestion, Stomach

1. Introduction

Understanding of digestive behavior of foods is useful for designing and developing foods with controlled digestibility (for example, foods that are easy to digest or from which nutrients can be easily absorbed) [1, 2]. In the human digestive tract, gastric digestion is important, because it directly affects the appetite. There is also social significance in designing foods with controlled gastric digestive behavior for promoting health. For example, when the appetite is not properly controlled by the gastric digestive behavior, obesity and metabolic syndrome might ensue [3, 4]. The stomach is linked to the brain through the vagus nerve. Bulky foods increase the stomach volume, and information on the increase in the volume of the stomach is transmitted to the related brain centers, which control the appetite [5, 6]. As an example of the influence of gastric distension on the appetite, it has been shown that bulky meals produced greater suppression of the appetite than less bulky meals with the same calorie content/nutrient composition [7, 8]. Aerated drinks suppress the appetite by producing a higher degree of stomach distension than non-aerated drinks [9].

Recently, for preventing obesity, a carbonated beverage containing low-methoxyl pectin (LM pectin) that expands and forms a bubble-containing gel in the stomach was developed. In the clinical trial, it was confirmed that participants who consumed this beverage felt satiety [10]. Appetite is suppressed by consuming this beverage and it is possible that obesity is also prevented. It was concluded that this drink might have the important effect of enhancing hunger suppression. If the volume of the bubble-containing gel is maximized and the expanded volume is sustained for longer, the stomach wall may be expected to expand to a larger extent and for a longer time and suppress hunger, which could possibly contribute to the prevention of obesity.

In the past, various in vitro simulators were developed for the evaluation of food digestion [11]. Among these, the human Gastric Digestion Simulator (GDS) [12-14]
enables *in vitro* evaluation of the digestive behavior of foods, considering the physical aspects, including peristalsis, and allows direct observation of the digestive process in real time. *In vitro* experiments using the GDS have confirmed that the bubble-containing gel formed by this beverage expanded to a significant degree and showed no disintegration even under the influence of peristalsis, and the volume decrease was suppressed [10]. It also confirmed that the gel volume changed dynamically. In this study, we investigated the factors that affect the volume of the bubble-containing gel and the volume-retention capacity using the GDS.

### 2. Materials and methods

#### 2.1 Materials

Japan Pharmacopoeia 1st fluid for disintegration test, pH 1.2, was purchased from Kanto Chemical Co., Ltd., Tokyo, Japan. For ingredients, such as LM pectin (United Foods International, Co., Ltd., Tokyo, Japan), citric acid monohydrate (Iwata Chemical Co., Ltd., Shizuoka, Japan), sodium benzoate (DSP Gokyo Food & Chemical Co., Ltd., Osaka, Japan), food additive grade ingredients were used. The product names of LM pectin used in this study were the followings; OF327C for pectin A, AYS407C for pectin B, OF445C for pectin C, OF463CSB for pectin D, OF805 for pectin E and LMSN325 for pectin F.

#### 2.2 Preparation of carbonated beverages containing pectin, citric acid and sodium benzoate

Solutions of pectin (Table 1) were prepared and diluted with carbonated water. A carbonated beverage containing LM pectin (Table 1) was prepared, filled in aluminum cans, sterilized at 80°C for 20 min, and used for the *in vitro* gastric digestion test. The concentrations of pectin and citric acid were set so as to obtain an appropriate viscosity as a beverage, and not gel, and also to obtain a proper buffering capacity of the beverage. The concentration of sodium benzoate used was the same as the concentration commonly used in beverages.

#### 2.3 Measurements of the physical properties

For measurement of the physical properties, samples without carbon dioxide gas were used. Samples measuring 80 mL in volume were placed in 40-mm dialysis tubing (Spectra/Per 1 Membrane fractionated molecular weight ~ 6,000-8,000) (Spectrum Laboratories Inc., Rancho Dominguez, CA) and dialyzed in 1600 mL of artificial gastric juice for 20 hours. The breaking stress and Young’s modulus of gel sample placed horizontally were measured using a rheometer (FUDOH Rheometer RTC) (Rheotech, Ltd., Tokyo, Japan). The physical properties were measured using a flat jig with a diameter of 1 cm. The descending speed of the jig and the strain rate were set at 1 cm/min and 100% ($n = 3$), respectively. An example of the stress-strain curve at the measurement of gel physical properties is shown in Fig. A.

#### 2.4 *In vitro* gastric digestion experiment

The *in vitro* gastric digestion experiment was conducted according to a previously reported method [14]. GDS was developed to quantitatively analyze and directly observe gastric digestion by simulating gastric peristalsis, gastric secretion, and gastric emptying similarly to

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**Table 1**: Composition of each carbonated beverage containing pectin.

| Sample name | Type of pectin | Origin | Esterification degree [%] | Amidation degree [%] | Pectin [w/v%] | Citric acid monohydrate [w/v%] | Sodium benzoate [w/v%] | Physical properties of gel without carbon dioxide gas |
|-------------|----------------|--------|---------------------------|---------------------|--------------|-------------------------------|-------------------------|---------------------------------------------------|
| Sample A-1  | A              | citrus | 34                        | 14                  | 0.50         | 0.20                          | 0.05                    | 5.3 [kPa] 16.6 [kPa]                                |
| Sample A-2  | A              | citrus | 34                        | 14                  | 0.50         | 1.00                          | 0.05                    | 1.6 [kPa] 9.1 [kPa]                                 |
| Sample B    | B              | citrus | 27                        | 20                  | 0.50         | 0.20                          | 0.05                    | 11.4 [kPa] 23.2 [kPa]                               |
| Sample C    | C              | citrus | 28                        | 18                  | 0.50         | 0.20                          | 0.05                    | 11.9 [kPa] 26.4 [kPa]                               |
| Sample D    | D              | citrus | 28                        | 10                  | 0.50         | 0.20                          | 0.05                    | 3.6 [kPa] 16.3 [kPa]                                |
| Sample E    | E              | apple  | 34                        | 15                  | 0.50         | 0.20                          | 0.05                    | 3.1 [kPa] 11.9 [kPa]                                |
| Sample F-1  | F              | apple  | 29                        | 18                  | 0.50         | 0.20                          | 0.05                    | 6.1 [kPa] 20.4 [kPa]                                |
| Sample F-2  | F              | apple  | 29                        | 18                  | 0.50         | 1.00                          | 0.05                    | 6.0 [kPa] 19.3 [kPa]                                |

*Each sample was adjusted to pH 4 with 1 mol/L of aqueous hydrochloric acid solution or 1 mol/L of aqueous sodium hydroxide solution.*
the human stomach. It consists of GDS vessels for digestion experiments, a temperature control system, a simulated gastric peristalsis system, and a secretion-emptying system. The GDS used in this test was designed by us.

2.4.1 Addition of the test beverage to artificial gastric juice

As the volume of gastric juice in the stomach in the fasting state, 50 mL of artificial gastric juice was transferred to an acrylic vessel (70 × 70 × 150 mm) or GDS. A funnel with a diameter of 120 mm was connected to a rubber tube with an inner diameter of 19 mm and length of 250 mm, corresponding to the inner diameter and length of the esophagus. Using this instrument, the test beverage (185 mL) cooled to 5°C or less was poured into an acrylic vessel or GDS at an inclination of about 45º for about 7 seconds. The pH values of gastric fluid in GDS after pouring the test beverage were 1.7-2.4 (10 min), 1.5-1.7 (30 min), 1.4-1.5 (60 min) and 1.2-1.4 (90 min), respectively.

2.4.2 Observation and evaluation of the digestion experiments

The speed of the simulated antral contraction waves (ACWs) was set at 2.5 mm/s, and their generation frequency was set at 1.5 cycle/min. The amount of gastric secretion and gastric emptying rate were set at 2.00 mL and 3.47 mL/min, respectively. The GDS was incubated at 37°C for 90 min.

The digestion process in the acrylic vessel or GDS was photographed and evaluated by analyzing the time-course of change in the volume of the bubble-containing gel. The mean volumes of the gel at 10, 30, 60 and 90 min after addition of the test beverage to the artificial gastric juice were calculated (n = 2). The gel was photographed from the front side of the acrylic vessel or GDS, and the volume of the gel at each time-point was calculated by the following Eq. (1) using the image analysis software, “Image J version 1.3.4.67.”

\[
\text{Gel volume [mL]} = \frac{A}{B} \times 300 \text{ mL} \quad (1)
\]

A: projected cross section of the gel; B: projected cross section of the 300-mL vessel

The gel volume retention rate was calculated using the following Eq. (2).

\[
\text{Gel volume retention rate [%]} = \frac{D}{C} \quad (2)
\]

C: after 10 min gel volume; D: after 90 min gel volume

3. Results and discussion

3.1 Effect of the type of pectin on the time-course of the gel volume

The effect of the type of pectin on the time-course of the gel volume was evaluated. As shown in Fig. 1, there was a difference in the gel volume at both 10 min after the bubble-containing gel formation and 90 min after gel formation depending on the type of pectin used (difference in the gel volume at 10 min after gel formation, maximum 22%; difference in the gel volume at 90 min after gel formation, maximum 27%). The initial gel volume at 10 min was smaller for the formulation prepared using LM pectin E, and the gel volume at 90 min was also less than that observed for the formulation prepared using LM pectin B/C/D. This result indicated that the initial gel volume influenced the gel volume at 90 min after gel formation. Differences in the gel volume and its digestive behavior may be affected by differences in the characteristics of the pectin raw material used (degree of esterification, degree of amidation, etc.). Since the pectin raw material parameters affect the gel physical properties, it is considered that the gel physical properties may affect the gel volume.

Images of the digestive behavior of the samples prepared using LM pectin B, E are shown in Fig. 2. When carbonated beverages containing LM pectin were dropped into the GDS filled with artificial gastric juice, generation of a bubble-containing gel was observed. In the case of the sample prepared using LM pectin B, a gel containing large bubbles was formed, whereas in the case of the sample prepared using LM pectin E, a gel...
containing fine bubbles was formed. It is thought that the more it forms a gel that can entrap larger bubbles, the larger the total volume of the bubble-containing gel becomes.

In this study, conditions were used that reduce the volume of the artificial gastric juice over time according to the actual digestive condition prevailing in the stomach. In the image, the liquid level decreased with decrease of the volume of the artificial gastric juice, and it was observed that the gel volume decreased with time due to peristalsis. Samples prepared using LM pectin B shrank while remaining tubular, whereas samples prepared using LM pectin E contracted spherically. It is considered that the difference in the physical strength of the bubble-containing gel may affect the rate of contraction of the gel.

3.2 Effect of the citric acid concentration in the formulation on the time-course of the gel volume

The effect of the citric acid concentration on the gel volume retention capacity was evaluated using the GDS. As shown in Fig. 3, there was a difference in the gel volume at both 10 min and 90 min depending on the difference in the citric acid concentration (difference in the gel volume at 10 min after gel formation, maximum 28%; difference in the gel volume at 90 min after gel formation, maximum 26%).

Images of the digestive behavior of two samples prepared using LM pectin A-1 and A-2 are shown in Fig. 4. Even when the same type of pectin was used, the shape of the bubble-containing gel differed significantly depending on the difference in the citric acid concentration; as the citric acid concentration increased, the bubbles, as well as the volume of the gel became smaller. The higher concentrations of buffering agents provide greater buffer capacity generally. As the buffering capacity increases with increasing concentration of citric acid, the pH increases when the sample is mixed with artificial gastric juice. When the formulation pH is constant and the citric acid concentration is high, the pH upon mixing the sample with artificial gastric juice becomes higher due to the high buffering capacity. In that case, the amount of gelation decreases and large air bubbles cannot be entrapped in the gel. Comparison of Figs. 2 and 4 reveals an agreement in the size of the bubbles and size of the gel volume, suggesting that factors influencing the bubble-containing gel volume are common. It is thought

![Fig. 2 Images of the digestive behavior of the samples prepared using LM pectin B and E in the GDS (effect of the type of pectin).](image)

![Fig. 3 Time-course of the gel volume (effect of the citric acid concentration).](image)
that the upper limit of the volume that allows the gel to
entrap the bubbles is reduced as a result of the decrease
in the ratio of sample that forms the gel due to the differ-
ence in pectin raw materials and the increase in citric
acid concentration.

3.3 Analysis of factors affecting the gel volume

Correlation analysis was carried out to analyze the fac-
tors affecting the gel volume and volume-retention
capacity. Since the degree of esterification and the
degree of amidation are derived only from the type of the
pectin raw material, samples prepared under identical
conditions (citric acid concentration: 0.2%) were used.
The volume-retention capacity of the gel volume was cal-
culated from the retention rate of the gel volume at 90
min with respect to that at 10 min.

3.3.1 Factors affecting the gel volume immediately after the reaction

As shown in Fig. 5, the gel volume at the 10-min evalu-
ation was correlated with the degree of esterification of
the pectin raw material (correlation coefficient: \( R = -0.73 \)). When the degree of esterification is small, the
non-dissociated carboxyl groups increase at low pH and
hydrogen bonding occurs [15]. Therefore, gelation is
likely to occur, and the proportion of the sample that gel-
ates is increased. As a result, it is considered that large
bubbles can be entrapped with the gel and the volume of
the gel will increase immediately upon the reaction. On
the contrary, when the degree of esterification is high, it
is considered that the ratio of the sample that forms a gel
is small, the amount of entrapped bubbles becomes
small, and the volume of the bubble-containing gel
decreases. The correlation between the gel volume at 10
min after gelation and the degree of amidation of pectin
was small (\( R = -0.16 \)).

Fig. 4 Images of the digestive behavior of the sample A-1 and A-2 in the GDS (effect of the citric
cacid concentration).

Fig. 5 Correlation analysis between each factor and the gel volume at 10 min after gel formation.
3.3.2 Factors affecting the gel volume retention capacity

As shown in Fig. 6 (a), the correlation with retention capacity of the gel volume and the degree of esterification was small (R= -0.29). On the other hand, as shown in Fig. 6 (b) (c) (d), the degree of amidation (R= 0.69) of pectin, the breaking stress (R= 0.65), and Young’s modulus (R= 0.63) affected the retention capacity of the gel volume. Amidated pectin is known to form stronger gels, especially at low pH [16], because hydrogen bonds are formed between amide groups [17]. The degree of amidation shows a relatively high correlation coefficient with the breaking stress (0.77) and Young’s modulus (0.66) (correlation figure not shown). There is a possibility that the degree of amidation indirectly affects the retention capacity of the gel volume through the breaking stress and Young’s modulus. When the degree of amidation is high, hydrogen bond formation increases. As a result, the crosslinking point of pectin increases, the structure of the gel becomes dense, and the strength of the gel increases, which can increase the retention capacity of the gel volume.

3.3.3 Summary of analysis of the factors

Below pH 3.5, LM pectin forms a gel even in the absence of calcium ions. In this study, it was found that the formulation conditions under which the degree of esterification of pectin and citric acid concentration were lower increased the size of the bubble-containing gel volume immediately upon reaction with gastric juice. It was also found that the retention capacity of the gel volume increased with a higher degree of amidation of pectin or a high breaking stress/Young’s modulus at the time of gelation. Therefore, the proportion of the sample participating in the gel formation is important for increasing the initial volume of the bubble-containing gel. The strength of the gel is important for retention of the gel volume.

It is necessary to consider the degree of esterification and amidation in order to maximize the volume of the bubble-containing gel and to increase the volume-retention capacity in the stomach. However, pectin is a naturally occurring polymer, and there are other differences, such as the molecular weight distribution and distribution of functional groups, so that there may also be other factors besides the degree of esterification and degree of amidation.

Although the experiment in this study was an in vitro experiment, it was possible to identify factors influencing the size of the bubble-containing gel and its volume-retention capacity in the stomach. This is the first case...
report of the factors of bubble–containing gels affecting on the gastric digestive behavior. As another result, we were able to find a pectin (Pectin B) that produced a larger–volume gel, with longer retention of the expanded volume, than the pectin (Pectin A) used in a previous study [10]. By using GDS, we were able to comprehensively identify an excellent type of pectin for gel formation. These findings are valuable for developing more effective drinks for the prevention of obesity.

It has been reported that the gastric emptying rate of foamed beverages is as slow as when producing more stable bubbles, and the feeling of satiety is also higher as the bubbles are more stable [9]. Since such a difference occurs even with non–gelled foam, there is a possibility that both the gastric emptying rate of the bubble–containing gel and the feeling of satiety are higher when the volume retention capacity is higher.

4. Conclusions

We attempted to identify factors that affect the volume of a bubble–containing gels and the volume–retention rate by evaluating their digestive behavior using a GDS. It was revealed that the degree of esterification of pectin and the citric acid concentration of the formulation affect the gel volume immediately upon reaction with artificial gastric juice. Furthermore, the degree of amidation of pectin and the physical properties of the gel (breaking stress and Young’s module) also affected the gel volume retention rate. For maximizing the volume of the bubble–containing gel and its volume retention rate when this beverage is consumed, it is necessary to consider the degree of esterification and amidation and the citric acid concentration in the formulation.

The factors affecting the gastric digestive behavior of a bubble–containing gel, including its volume and retention, were evaluated for the first time. These results are expected to make it possible to optimize the gastric digestive behavior of beverages that form bubble–containing gels in the stomach.

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Fig. A  An example of the stress–strain curve of gel without carbon dioxide gas (Sample A–1) used for the determination of the physical properties shown in Table 1.
気泡含有ゲルの胃消化挙動に及ぼすペクチン種およびクエン酸濃度の影響：ヒト胃消化シミュレーターを用いた評価

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近年, 胃内で気泡含有ゲルを形成し膨張することにより, 少ない摂取カロリーで空腹感を抑制できる飲料が開発された。この飲料の空腹感抑制効果を高めるには, 胃壁をより大きく, 長い時間伸展させるように, 大きな体積の気泡含有ゲルを形成し保持できる飲料の最適な作製条件を明らかにすることが重要である。そこで, \textit{in vitro} で胃における食品の消化挙動を評価できる胃消化シミュレーターを使用し, 気泡含有ゲルの体積やその保持能に影響する因子を評価した。その結果, ペクチンのエステル化度やクエン酸濃度が胃液との反応直後のゲル体積に影響することがわかった。また, アミド化度やゲル物性がゲル体積の保持能に影響することがわかった。気泡含有ゲルの体積を最大化させ体積保持能を高めるためには, ペクチンのエステル化度やアミド化度, 製剤中のクエン酸濃度を考慮する必要がある。
