Note

Formulation and Evaluation of a Satiety-inducing Carbonated Beverage that Forms a Bubble-containing Gel in the Stomach

Takashi Domoto1,2*, Hiroyuki Kozu2, Marie Yamaji1, Takuto Takei1, Kazuto Nishijima1, Kiichi Matsudo1, Yutaka Mizuma1, Kazutaka Saiso1, Isao Kobayashi1 and Sosaku Ichikawa2*

1Taisho Pharmaceutical Co., Ltd. 3-24-1 Takada, Toshima-ku, Tokyo 170-8633, Japan
2Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8572, Japan
3Food Research Institute, NARO, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

Received October 28, 2017; Accepted February 27, 2018

We developed a beverage that forms a gel containing gas bubbles in the stomach and induces satiety. In a preliminary experiment, we confirmed that when a carbonated beverage containing any one of three types of ionic polysaccharides was mixed with artificial gastric juice, it resulted in the formation of a gel containing gas bubbles. Among the three types of polysaccharides referenced above, low methoxyl pectin (LM pectin) was identified as being the optimal for preparation of the test beverage in this study. Both static evaluation using a vessel and dynamic evaluation using the Gastric Digestion Simulator (GDS) revealed that the volume of the bubble-containing gel remained relatively stable with time. Presence of the bubble-containing gel in the stomach following consumption of the carbonated beverage containing LM pectin was confirmed in a clinical Magnetic Resonance Imaging (MRI) study. Consumption of this test beverage resulted in a greater increase of the intragastric volume than consumption of the same amount of water. Moreover, in the satiety questionnaire, the subjects reported a higher degree of satiety following consumption of the beverage than following consumption of an equal amount of water. These results indicate that when this test beverage, a carbonated beverage containing LM pectin that forms a bubble-containing gel in the stomach, is consumed, the stomach becomes distended, inducing a feeling of satiety.

Keywords: pectin, gel, beverage, MRI, stomach, satiety, obesity

Introduction

Excessive accumulation of visceral fat is a risk factor for lifestyle diseases such as type 2 diabetes mellitus, dyslipidemia and hypertension; therefore, its prevention is considered to be of significant clinical consequence. As one of the means for improving lifestyle habits, it is necessary to control the caloric intake by diet therapy; however, controlling an individual’s appetite is not always easy. Gastric distension could be an effective and safe means for suppressing appetite; information on the increase in the volume of the stomach is transmitted to the related brain centers through the vagus nerve (Camilleri, 2015; Williams et al., 2016). Obesity treatment based on this mechanism, by placing a balloon in the stomach, has been reported (Saber et al., 2017). As an example of the influence of gastric distension on the appetite, it has been shown that bulky meals produced greater suppression of appetite than less bulky meals with the same calorie content or nutrient composition (Melnikov et al., 2014; Peters et al., 2015). Aerated drinks suppress the appetite by producing a higher degree of stomach distension than non-aerated drinks (Murray et al., 2015). Based

*To whom correspondence should be addressed.

E-mail: Takashi Domoto;
domoto.takashi.xd@alumni.tsukuba.ac.jp
Sosaku Ichikawa; sosaku.ichikawa.fn@u.tsukuba.ac.jp
on a previous report that some ionic polysaccharides have the
property of gelling in response to exposure to a suitable pH
(Itoh et al., 2006), attempts have been made to induce satiety
using beverages that, when consumed, would result in gel
formation in the stomach (Hoad et al., 2004).

We developed, for the first time, a carbonated beverage
that, when consumed, forms a bubble-containing gel in the
stomach. Since this beverage not only shows gelation, but also
expands in the stomach by trapping carbonic bubbles, it is
expected to induce satiety by causing distension of the
stomach.

Recently, a novel in vitro gastric digestion device focused
mainly on evaluation of the physical aspects of digestion in the
human stomach was developed (Gastric Digestion Simulator
(GDS) (Kozu et al., 2010, 2014, 2017)). The GDS enables
evaluation of digestion considering the physical aspects of
digestion, including peristalsis, and allows direct observation
of the digestive process in real time. We investigated the
digestive behavior of the carbonated beverage developed by us
for this study by in vitro tests, including static evaluation
performed using a vessel, and dynamic evaluation performed
using the GDS. Furthermore, we also conducted in vitro
evaluation by Magnetic Resonance Imaging (MRI), to confirm
the gel formation in the stomach and distension of the stomach
following consumption of this beverage. Furthermore, we used
the satiety questionnaire to confirm whether the test beverage
indeed induced satiety.

Materials and Methods

Materials. Janum Pharmaceuticals 1st fluid for disintegration
test, pH 2.1, was purchased from Kanto Chemical Co., Ltd. The
ingredients such as low methoxyl pectin (LM pectin), high
methoxyl pectin (HM pectin), sodium alginate, decacyl-type
gelatin gum, carrageenan, citric acid, and xanthan gum
were used.

Preparation of the test beverage. Solutions of ionic
polysaccharides (Table 1) were prepared, diluted with purified
water or carbonated water, and immediately used for the in
vitro gelation tests. A carbonated beverage containing LM
pectin (Table 1) was prepared, filled in aluminum cans,
stereilized, and used for the in vitro gastric digestion test. The
carbonated beverage containing HM pectin (Table 1) was prepared,
filled in glass bottles, and immediately used for the in
vitro gelation test. The concentration of sodium benzoate used was the same as
that commonly used in beverages.

In vitro gelation test. The test beverage (100 mL) cooled to
5°C or lower was added dropwise to artificial gastric juice
(100 mL) heated to 37°C, and the state of the solution was
evaluated. The expansion rate of the gel was calculated using
the following formula:

\[ \text{Expansion rate} = \frac{V_{\text{expanded}} - V_{\text{initial}}}{V_{\text{initial}}} \times 100\% \]

Table 1. Composition and characteristics of each test beverage

<table>
<thead>
<tr>
<th>Ingredients [w/v%]</th>
<th>( \kappa )-Carrageenan</th>
<th>Xanthan gum</th>
<th>High methoxyl pectin</th>
<th>Low methoxyl pectin</th>
<th>Sodium alginate</th>
<th>Gelatin gum (Decacyl type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysaccharide</td>
<td>0.05</td>
<td>0.05</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.05</td>
</tr>
<tr>
<td>Citric acid monohydrate</td>
<td>0.05</td>
<td>0.05</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Aqueous solution</td>
<td>Non-carbonated</td>
<td>Carbonated</td>
<td>Non-carbonated</td>
<td>Carbonated</td>
<td>Non-carbonated</td>
<td>Carbonated</td>
</tr>
<tr>
<td>Properties after mixing with artificial gastric juice</td>
<td>Liquid</td>
<td>Liquid</td>
<td>Liquid</td>
<td>Liquid</td>
<td>Gel</td>
<td>Expanded gel</td>
</tr>
</tbody>
</table>

The test beverage pH was adjusted with 1 mol/L of aqueous hydrochloric acid solution or 1 mol/L of aqueous sodium hydroxide solution (test beverage containing gelatin gum gelled at pH 4 and adjusted to pH 5).

One hundred mL of a test beverage cooled to 5°C or lower was added dropwise to 100 mL of artificial gastric juice at 37°C. After incubation at 37°C for 10 minutes, the condition was observed.
A, total volume; B, test beverage volume; C, volume of artificial gastric juice

The viscosity at the time of preparation was measured at 20°C, using an automatic micro viscometer (AMVn) (Anton Paar Co., Ltd., Graz, Austria).

**In vitro gastric digestion experiment**

(1) Addition of the test beverage to artificial gastric juice

As the volume of gastric juice in the stomach in the fasting state (Ricci et al., 1993), 50 mL of artificial gastric juice was transferred to an acrylic vessel (70 × 70 × 150 mm) of the 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 the acrylic vessel or the GDS at an inclination of about 45° for about 7 s.

(2) Observation and evaluation of the digestion experiments

Static evaluation was performed by incubating the acrylic vessel at 37°C for 90 min. The speed and generation frequency of the Antral Contraction Wave (ACW) of the GDS was set to 2.5 mm/s and 1.5 cycles/min, respectively (Kozu et al., 2010, 2014). In order to simulate the effects of gastric juice secretion and emptying, a GDS equipped with a supply and emptying system for artificial gastric juice (Kozu et al., 2017) was used. Based on a previous report as reference (Marciani et al., 2001; Murray et al., 2015), 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/GDS was photographed and evaluated by analyzing the time-course of changes in the volume of the bubble-containing gel. The mean volumes of the gel were calculated at 10, 30, 60 and 90 min after addition of the test beverage to artificial gastric juice (n = 4). The gel was photographed from the frontal aspect of the acrylic vessel of GDS, and the volume of the gel at each time-point was calculated by the following formula using the image analysis software, “Image J version 1.3.4.67.”

\[
\text{Gel volume (mL)} = \frac{A}{B} \times 300 \text{mL} \quad \text{⋯⋯Eq. 2}
\]

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

**Clinical trial of single consumption** In order to confirm that the formation and expansion of the gel occurred not only *in vitro*, but also in the human stomach, a clinical trial of a single administration of the test beverage was conducted.

(1) Participants

This study was conducted with the approval of the Shiba Palace Clinic Ethics Review Committee obtained on January 28, 2016. In compliance with the principles of the Declaration of Helsinki, the study was conducted in conformity with ethical guidelines (Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare) on medical science research in humans. After providing a clear explanation about the study to the participants, written consent was obtained from each subject prior to his/her participation in this study. Healthy adults aged 20 years old to under 65 years old were targeted, and those who reported feeling hungry just before taking the control/test beverage, with the degree of hunger falling under category B or C described below, were selected as the study subjects: A, feels very hungry; B, feels hungry; C, feels a little hungry. A total of 6 subjects were enrolled. We excluded persons with conditions (including those taking drugs, those with a history of gastrointestinal disorders, etc.) that could affect the test results.

(2) Test beverage and control

Carbonated water containing LM pectin (Cobra Support® apple flavor) was used as the test beverage, and water (Livita®natural water) was used as the control. The ingredients in Cobra Support® were high-fructose corn syrup, indigestible dextrin, pectin, an acidulant, flavor, sodium benzoate, and a sweetener (acesulfame K or sucralose). It contained 4.7 g of dietary fiber (0.5 g of pectin, 4.2 g of indigestible dextrin) and 6.5 g of carbohydrate.

(3) Test method

This study was conducted in February 2016 as a randomized, open-label, crossover (consumption of the test and control beverages) study. Each patient consumed the test and control beverages once each. The interval between the first period and second period was at least 6 d.

The MRI examination was conducted immediately before, and 10, 30 and 60 min after each subject consumed the control/test beverage. The MRI images were acquired during breath-holding by the subject. Transverse images of the stomach were acquired at a slice thickness of about 3 to 5 mm. The MRI measurement conditions were optimized and set by MRI measurement of a solution in which an acidic solution and the control (water) or test beverage were mixed in advance.

The satiety questionnaire was administered 10, 30 and 60 min after each subject consumed the control/test beverage.

(4) Evaluation method

The primary endpoint was the formation/non-formation of a bubble-containing gel in the stomach following consumption of the control/test beverage. From the imaging data obtained 10 min after consumption of the control/test beverage, the image with the maximum cross-sectional area of the stomach was identified and the investigator judged the presence/absence of bubble-containing gel in that image. A representative MRI image is shown in Fig. 1. The secondary endpoints were as follows: changes in the internal volume of the stomach, changes in the volume of the bubble-containing gel in the stomach, changes in the sum of the volumes of the intragastric liquid part plus bubble-containing gel part, and the responses to
the satiety questionnaire. Using an image analysis software (medical image analysis application OsiriX), the areas of the gel part, the liquid part and the gas part in all of the photographed images were calculated, and the internal volumes of the stomach were calculated by the integration method. The response options for the items on the satiety questionnaire were “satisfied,” “somewhat satisfied,” “slightly satisfied,” “same as before drinking,” and “hungry before drinking.”

5) Statistical analysis
For the presence/absence of the bubble-containing gel in the stomach and responses to the satiety feeling questionnaire, the frequencies were counted. For the internal volume of the stomach, basic statistics were calculated for each measured value and for the change in volume from immediately before consumption of the control/test beverage. One-specimen t test was used for comparison of the volumes with those just before consumption of the test beverage, and the two-specimen t test for comparison with the volumes after consumption of the control. The significance level was set at 5% (two-sided), and the SAS Ver. 9.1.3 software was used for the analysis.

Results and Discussion
Formulation of the carbonated beverage that forms a bubble-containing gel upon contact with artificial gastric juice
Aqueous solutions or carbonated aqueous solutions containing any of six representative ionic polysaccharides were prepared, and their bubble-containing gel-forming capabilities upon being mixed with artificial gastric juice were evaluated. The results revealed formation of a bubble-containing gel when a carbonated aqueous solution of LM pectin or sodium alginate or gellan gum was mixed with artificial gastric juice (Table 1). Gel formation following mixing of an aqueous solution containing LM pectin with gastric juice has been reported previously (Itoh et al., 2006; Hoad et al., 2004). In this study, it was found for the first time that a carbonated beverage containing LM pectin expands to form a bubble-containing gel. This is considered to be attributable to vaporization of carbon dioxide gas during formation of the gel when the solution is mixed with artificial gastric juice. Since solutions of carrageenan (functional ionic group, sulfate), xanthan gum (ratio of acidic sugar small) and HM pectin (more than half of the carboxyl groups methoxylated) did not show gel formation upon being mixed with artificial gastric juice, the ratio of carboxyl groups is considered to be important for the pH-responsive gel formation. An example of the differences in the reactions of the solutions is shown in Fig. 2.

The influence of the polysaccharide concentration on the expansion rate of the gel and the viscosity of the beverage at the time of preparation was examined for the three polysaccharides that allowed the carbonated solutions to form a bubble-containing gel upon being mixed with gastric juice. The results (Fig. 3) revealed that the gels formed with solutions containing LM pectin and gellan gum showed marked expansion even at low concentrations of <0.1% (w/v); on the other hand, addition of alginic acid up to 0.3% (w/v) did not induce gel formation, and even at higher concentrations at
Carbonated Beverage that Forms Bubble-containing Gel in the Stomach

which gel formation was induced, the expansion rate of the gel was low (Fig. 3a). This gelation concentration of alginate was consistent with previous reports (Itoh et al., 2006; Hoad et al., 2004). We found, for the first time, that the rate of expansion of the bubble-containing gel increased with the polysaccharide concentration. In regard to the viscosity of the solution at the time of preparation, the solution containing gellan gum showed a higher viscosity than the solution containing LM pectin at the same concentration; therefore, gellan gum was considered as being unsuitable, while LM pectin was selected as the most suitable polysaccharide for preparation of the test beverage for this study (Fig. 3b). We chose a 0.5% concentration of LM pectin, because it yielded a moderate viscosity of the beverage and the maximum expansion rate of the gel in the stomach.

In vitro gastric digestion experiments To examine how the bubble-containing gel changed with time, we performed two types of tests, one to test the stability of the gel and the other to test its ability to withstand digestion. In order to evaluate the stability of the bubble-containing gel, we conducted static evaluation using a vessel, and confirmed that the gel expansion was stable and not transient, and that the strength of the gel was sufficient to allow the increased volume
T. Domoto et al.

To be stably maintained (Fig. 4a). Ten minutes after being mixed with artificial gastric juice, the sample increased in volume by about 1.3-fold, and the volume was maintained at 75% of the maximal volume even after 90 min (Fig. 5). In order to evaluate the ability of the bubble-containing gel to resist digestion in the human stomach, we conducted dynamic evaluation using the GDS, considering the influence of peristalsis. The gel gradually shrank when the bubbles ruptured from the bottom end of the bubble containing gel (Fig. 4b). However, despite the steady decrease of the gel volume, the volume was maintained at 40% of the maximal volume even after 90 min (Fig. 5). Thus, the bubbles ruptured in the part of the gel that was directly affected by peristalsis. The gel gradually shrank from the outside, with disappearance of the bubbles. This digestion behavior was different from that of tofu as a typical solid food (Kozu et al., 2014). In general, solid foods are chewed and broken into small fragments, and then swallowed, to reach the stomach; the fragments are then digested into fine particles in the stomach. On the other hand, bubble-containing gels form one large lump in the stomach, which gradually shrinks in size with time.

**Clinical trial of single consumption** We enrolled a total of 6 subjects. The test was carried out as planned in all of the subjects, and data from all the cases were included in the analysis. There were 2 males and 4 females, with an average age of 48.0 years (26-57 years) and an average BMI of 21.67 (18.9-24.8).

The formation of the bubble-containing gel following consumption of the test beverage was confirmed in all the subjects, whereas it was found in none of the subjects following

---

**Fig. 4.** Time-course of changes in the gel images in the static evaluation using a vessel and dynamic evaluation using the GDS. (A) Image of carbonated water containing LM pectin left standing for 90 minutes after it was mixed with artificial gastric juice. (B) Image of carbonated water containing LM pectin digested with GDS for 90 minutes after it was mixed with artificial gastric juice. Each scale is plotted on the GDS vessel at 50-mL intervals.

**Fig. 5.** Bubble-containing gel volume (n = 4) in the static evaluation using a vessel and dynamic evaluation using the GDS. From the image obtained from the frontal aspect of the vessel, the gel volume was calculated using the following formula.

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

A, projected cross section of gel; B, projected cross section of the 300-mL of vessel.
consumption of water (control).

The mean gastric internal volumes (bubble-containing gel part plus the liquid part plus the gas part) immediately before and 10 min after consumption of the control/test beverage are shown in Fig. 6. At 10 min after consumption of the test beverage, the intragastric volume increased by 407 ± 211 mL (mean ± standard deviation) \( (p = 0.005) \), and at 10 minutes following the consumption of water, it increased by 190 ± 116 mL \( (p = 0.010) \). The difference in the degree of increase was 217 mL, although it did not reach statistical significance \( (p = 0.052) \). No significant difference in the intragastric volume as compared to that recorded before the consumption was observed at either 30 or 60 min after water consumption. The total volume of the gel and liquid increased by 182 mL at 10 min after consumption of the test beverage relative to that after consumption of water, and the difference was significant \( (p = 0.043) \). In this study, we confirmed formation of the bubble-containing gel in the stomach following consumption of the carbonated beverage containing LM pectin by MRI for the first time, even though formation of air bubbles in the stomach has been demonstrated previously (Murray et al., 2015).

The satiety questionnaire was administered to the same 6 subjects to evaluate their feeling of hunger at 10 min after consumption of the control/test beverage, that is, at the start of the MRI examination. The feeling of satiety tended to be greater after consumption of the test beverage as compared to that after consumption of water (Fig. 7). The responses to the satiety questionnaire showed that after consumption of the test beverage, all the subjects selected the response of “slightly satisfied” or better at every time-point of evaluation. The percentage of subjects indicating “somewhat satisfied” at 10 minutes after consumption of the test beverage was 83%, whereas that after consumption of water was 33%. Thus, the test beverage induced a higher degree of satiety than water.

Satiety was induced in all the subjects (there were no subjects who said they were as hungry after consumption of the test beverage); therefore, it was concluded that the carbonated beverage containing LM pectin had the ability to induce satiety.

This is the first report showing that formation of a bubble-containing gel in the stomach following intake of a carbonated beverage induced satiety, although induction of satiety by non-carbonated gel-forming beverages has been reported previously (Hoad et al., 2004). This is thought to be due to the fact that the carbonated beverage containing LM pectin produced a greater degree of gastric distension than water.

The beverage evaluated in this study also contained high-fructose corn syrup and indigestible dextrin. Fructose and glucose increase the blood glucose level, which can also lead to the inducement of satiety. It has been reported that indigestible dextrin is broken down by intestinal bacteria in the large intestine to produce short-chain fatty acids, which induce satiety (Hobden et al., 2015). Carbonated water is also known to induce satiety (Wakisaka et al., 2012). It has been reported that a bulky diet induces a feeling of fullness as compared to a non-bulky diet even for the same calorie content or nutrient composition (Melnikov et al., 2014; Peters et al., 2015). Thus, it would seem that gastric distension plays a role in the induction of satiety.

Beverages containing pectin and alginic acid have been reported previously as being effective for inducing satiety (Peters et al., 2011; Wanders et al., 2014). Several reports have confirmed that it is gelation of the polysaccharide contained in the beverage that induces satiety following consumption of these beverages. Using carbonated beverages containing pectin formulations enables higher-volume gel formation, which causes a higher degree of distension of the gastric wall, inducing satiety.
Based on these results, we propose that consumption of the test beverage developed by us in the fasting state would induce satiety, by causing distension of the stomach. Therefore, the carbonated beverage containing LM pectin developed by us may be a highly useful supplementary food for reducing the risk of lifestyle diseases.

Conclusions

We demonstrated that carbonated beverages containing LM pectin showed gel formation and expansion upon being mixed with artificial gastric juice. The volume of the bubble-containing gel remained relatively stable in the in vitro gastric digestion experiment. The MRI study confirmed formation of the bubble-containing gel in the stomach following consumption of the carbonated beverage containing LM pectin. The results of the satiety questionnaire also revealed that the beverage induced a higher level of satiety than consumption of water at the same volume.

References