Development and Evaluation of a Beverage that Forms Bubble-Containing Gel in the Stomach

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Takashi Domoto

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Takashi Domoto

Contents

Abbreviations

ACW	Antral contraction wave
BMI	Body mass index
GDS	Gastric digestion simulator
c-GDS	Continuous-type gastric digestion simulator
HM pectin	High-methoxyl pectin
MRI	Magnetic resonance imaging
LM pectin	Low-methoxyl pectin

CHAPTER 1. GENERAL INTRODUCTION

1.1. Efforts for Obesity Prevention

In recent years, extension of healthy life expectancy (period in which daily life can be lived without being restricted due to health problems) has become a major issue. In Japan, the difference between average life expectancy and average healthy life expectancy is about 9 years for men and about 12 years for women, and the greater the difference between average life expectancy and average healthy life expectancy, the greater the need for home care and medical care becomes. Closing the gap between the two averages is important from the standpoint of individual well-being, curbing home care and medical expenses, and responding to the decline in the size of the working population and the shortage of care workers.

The Japanese government has launched “Health Japan 21” as a new national health promotion campaign in which the people can take the initiative. Based on the idea of extending healthy life expectancy and reducing health disparities, five basic directions and the goals for each direction were set by the Ministry of Health, Labor and Welfare of Japan (Ministry of Health, Labor and Welfare HP (i)). The goals include items related to obesity, e.g., “Prevention of lifestyle-related diseases” and “Eating habits, exercise, rest, drinking, smoking, improving lifestyle and the social environment related to oral health”, and furthermore, as a target value for the countermeasure against obesity, for example, a numerical value of “less than 28% of obese males (BMI 25 or more) in 20’s to 60’s” is set.

A person with a body mass index (BMI = (weight [kg])/(height [m])^2) of 25 or more is regarded as “obese”. Fat distribution has recently come to be regarded as important, and visceral-fat-type obesity is a high-risk form of obesity that tends to be accompanied by health problems. Obesity is defined as a medical condition that requires weight loss when a person who has been diagnosed as obese has or is expected to have a health disorder caused by or related to obesity (Miyazaki *et al.,* 2018).

Excessive visceral fat accumulation is a risk factor for lifestyle-related diseases such as hyperglycemia, dyslipidemia, and hypertension due to type 2 diabetes, and it is one of the elements of metabolic syndrome that develops by combining two or more of these diseases. Obesity causes these lifestyle-related diseases, and it exacerbates them. As a result, blood vessels may become brittle or damaged, causing arteriosclerosis and progressing to serious illnesses such as myocardial infarction and stroke. Thus, obesity needs to be prevented in order to extend the healthy life expectancy.

In recent years, obesity has been found to cause the various other complications besides lifestyle-related diseases (The Japan Foundation for Aging and Health HP (ii)). Obesity imposes an excessive load on the hip and knee joints that causes osteoarthritis and low back pain. Obesity is a risk factor for breast cancer in postmenopausal women and is a negative prognostic factor for malignant disease. Obesity is also a risk factor for colorectal cancer, and it is a risk factor for a decline in activities of daily living in women.

In order to achieve the numerical targets of "Healthy Japan 21", the Japanese government has implemented a variety of activities, including the introduction of a new concept of obesity and metabolic syndrome prevention, and efforts to easily adjust the amount of energy in meals. The national government, local governments, and private companies are working on legislation, establishing systems, raising awareness, and implementing measures. For example, local governments cooperate with employee cafeterias to support food environment development, such as the development of healthy menus and the transmission of health information. In addition, companies are recruiting participants in health seminars from among employees who have been advised to obtain specific health guidance (Ministry of Health, Labor and Welfare, Japan HP (iii)).

Thus, great efforts have been made nationwide, and an effective method of preventing obesity is desired.

1.2. Factors that Affect Obesity

There are three causes of obesity (Yoshimatsu *et al*., 2001). The first cause is metabolic factors. In addition to genetic effects, metabolic conditions related to diabetes, such as decreased insulin sensitivity and abnormal sugar and lipid metabolism, affect obesity. The second cause is environmental factors, including social factors such as changes in dietary habits, car-oriented society, day-night reversal, and stress. The third cause is behavioral factors, including individual behaviors such as overeating, snacking, rapid eating, lack of exercise, and motivation, and behavioral factors are the easiest cause to approach in terms of prevention. Obesity can be prevented easily by changing the daily behavior of individuals, without relying on medical institutions.

There are two ways to combat obesity: reduce energy intake and increase energy consumption. Reducing energy consumption requires a review of eating habits, and increasing energy consumption requires exercise promotion. It is difficult to continuously exercise with great effects due to time constraints and physical burdens. The individual's appetite is the main obstacle to improving dietary habits, such as overeating, snacking,

unbalanced eating, and rapid eating. Since the desire to eat high-calorie foods and to eat more is a human instinct from the hunting era when the food supply was inadequate, few effective methods of appetite control are available at present.

1.3. Factors that Affect Appetite

Various factors are known to affect appetite. The hypothalamus is the part of the brain that controls appetite. The hypothalamus controls circadian rhythms, sleep, and body temperature as well as feeding behavior, and all of them also influence the hypothalamus. Appetite control in the hypothalamus is influenced by the five senses, i.e., by vision, olfaction, hearing, touch, and taste in the cerebral neocortex and limbic system, by emotions, and by memory. In addition, changes in blood glucose levels in peripheral organs caused by food intake, gastrointestinal hormone secretion, stomach wall stretching, etc., also affect appetite via the vagus nerve (Ueno *et al*., 2015). The factors that affect appetite and their relationships are summarized in Fig. 1.1.

Drugs that control appetite by acting directly on the hypothalamus have been developed, but risks of various side effects have also been reported (Philip *et al*., 2010). The five senses and circadian rhythms are difficult to control because of the various factors involved such as the sight and smell of food, meal times, and life style. In addition, controlling blood sugar and gastrointestinal hormones may cause side effects involving metabolic organs.

On the other hand, one of the factors that affects appetite shown in Fig. 1.1, i.e., stretching the stomach wall by an increase in the volume of gastric contents, is transmitted to the hypothalamus through the vagus nerve and acts to suppress appetite, so the risk of adverse effects is low (Wang *et al*., 2007; Camilleri *et al*., 2015; Williams *et al*., 2016). Moreover, if the stomach wall could be stretched with a low-calorie food, it would provide an effective method of preventing obesity by suppressing appetite.

1.4. Methods of Stretching the Stomach Wall

Placing a balloon in the human stomach to suppress appetite by stretching the stomach wall is being used as a method of treating obesity (Horner *et al*., 2011; Saber *et al*., 2016). However, this method is expensive because it involves a surgical operation, and it is invasive and is very stressful physically on the body.

On the other hand, ingestion of foods that are bulky and retain their volume in the human stomach for a relatively long time has been reported as a method of suppressing appetite by stretching the stomach wall (Rolls *et al*., 2000). For example, a method of

suppressing appetite by consuming low-calorie, bulky foods, such as dishes prepared with many vegetables, is being widely used (Rolls *et al*., 2009). However, since it takes time to prepare such dishes for every meal, it is not an appetite suppression method that can be easily and continually utilized.

Since beverages can be easily consumed at any time, they can be described as suitable foods for controlling appetite. Thus, research has been conducted on appetite-suppressing beverages, and examples of them are summarized in Table 1.1. Carbonated water is an example of a beverage that has been reported to be useful as a means of appetite control (Wakisaka *et al*., 2012); however, shortly after ingestion, the bubble component that contributes to extending the stomach wall is released in the form of a burp, and the appetite-suppressing effect cannot be exerted continuously.

A high viscosity beverage containing a large amount of polysaccharide has been reported to be effective in suppressing appetite (Marciani *et al*., 2001), and beverages containing sodium alginate increase gastric residence time and suppress appetite (Hoad *et al*., 2004), but because of their high viscosity, they cannot be consumed easily.

Beverages that foam and contain numerous air bubbles are known to greatly expand the stomach wall and suppress appetite more than beverages that do not foam (Murray *et al*., 2015). However, since it is necessary to prepare the foam just before consuming, it is difficult to take such beverages anywhere, and they are inconvenient to use.

Thus, although research has been conducted on beverages that suppress appetite, no beverages that are ingestible, persistent, and convenient have been proposed.

1.5. Application of Pharmaceutical Technology to Food Technology as a means of Appetite Control

In the pharmaceutical industry, gastroretentive formulations, mucoadhesive formulations, and colon-targeting formulations have been studied as a means of controlling the absorption of pharmacological components in the digestive tract (Ogawara, 2009). Techniques for controlling drug retention have also been studied, and they may be used to develop beverages that stretch the stomach wall.

Formulations that exploit polysaccharide gelation by gastric acid have also been developed, e.g., there is a report of using LM pectin to achieve sustained release of a drug by taking advantage of its gelation by gastric acid (Itoh *et al*., 2006).

On the other hand, formulations have also been developed in which the physical properties of the formulation itself, not controlled release of pharmacological components in the stomach, act on the stomach. For example, the sodium alginate in Gaviscon™, a gastric drug, gels in response to a decrease in pH caused by gastric acid (Strugala *et al*., 2012). Carbon dioxide is generated from sodium hydrogen carbonate and forms bubbles, and a gel containing bubbles forms and floats to the upper part of the stomach. As a result, the gastric contents are coated with a gel containing air bubbles, which physically prevents the low-pH gastric acid from entering the esophagus, and thus prevents heartburn and reflux esophagitis (Malmud *et al*., 1979; Prajapati *et al*., 2013). Thus, much research and development has been conducted on formulations that produce a bubble-containing gel that floats in the stomach. Pectin, as well as sodium alginate, has been used as a gelling agent (Havelund *et al*., 1997), and guar gum, xanthan gum, carrageenan, locust bean gum and similar substances have been used in combination with alginic acid (Kapadia *et al*., 2007). Their formulations are summarized in Table 1.2.

Most gelling components contained in formulations that form a gel in the low-pH environment of the stomach are ionic polymers, such as ionic polysaccharides. Since the stomach is made highly acidic by the gastric juice it secretes, the formulations take advantage of the fact that the ionic polymer contained in the preparation gels under low-pH conditions. Some gelling ingredients cannot be used in food, but many naturally derived polysaccharides can be used in food. If a large bubble-containing gel produced by a beverage is formed in the stomach, it may be useful as a means of inducing satiety.

1.6. Methods of Evaluating a Gel under Human Gastric Conditions

Research and development of beverages that produce a bubble-containing gel in the stomach requires evaluation of the formation of the bubble-containing gel in the stomach and its persistence over time, and devices are available to perform *in vitro* tests. Clinical trials allow investigation of digestive behavior in the actual human stomach, but experiments on many subjects are necessary to obtain highly accurate results. Moreover, testing is relatively expensive and stressful for the subject, which makes it difficult to systematically evaluate many samples.

In past clinical trials, human stomach contents have been evaluated by various detection methods. A method of observing and evaluating human gastrointestinal tract dynamics radiographically after ingesting radioisotope-labeled food labeled has been used since the 1970s. Because it entails such problems as requiring special equipment and the complexity

of handling radioactive materials, it is not commonly used at present (Kawasaki *et al*., 2002). An ultrasonic method has been tried, but quantitative analysis is difficult. By contrast, the magnetic resonance imaging (MRI) method imposes only a small physical burden on the subject and the data obtained can be analyzed quantitatively. For those reasons, in recent years it has frequently been used to evaluate the state of stomach contents and their retention (Marciani *et al*., 2001; Murray *et al*., 2015).

I*n vitro* tests allow more reproducible experimental conditions that simulate the human gastric environment. However, the digestive behavior of the human stomach on food cannot be perfectly reproduced *in vitro*.

A static test that involves the use of a container such as a flask is one of the *in vitro* methods that can be applied to a bubble-containing gel. This method makes it possible to use artificial gastric juice to conduct a test that simulates the chemical environment in the stomach, such as the pH conditions. Because this method does not require any special equipment, it makes it easy to evaluate the behavior of a bubble-containing gel.

On the other hand, because of the peristalsis that occurs *in vivo*, such simple test methods cannot properly simulate the physical environment of the human stomach, and thus it is difficult to evaluate the changes in volume of a bubble-containing gel in the stomach, which are affected by peristaltic movements.

1.7. Objective of this Study and Organization of this Thesis

1.7.1 Objective of this study

Since obesity leads to lifestyle-related diseases, it is important to prevent it. Various factors influence obesity, but the focus of this study was “appetite control”, which can be approached through food.

There are various ways of controlling appetite. Among of them, stretching the stomach wall is safer than other methods, and it can be achieved by using the physical properties of the food. A beverage that affect appetite by stretching stomach wall were investigated in this study.

The appetite suppression methods that have been reported in the past to stretch the stomach wall by consuming beverages have entailed some challenges. The beverages had poor ingestibility, persistence, or convenience. If a beverage that has excellent ingestibility, persistence, and convenience and stretches the stomach wall could be developed, it would be able to assist in obesity prevention.

Therefore, as a method for achieving ingestibility, persistence, and convenience, application of the gastric gelling preparation technique used in the pharmaceutical industry was considered. Ingredients frequently used in such formulations have included ionic polysaccharides, which can also be used in food. It might be possible to develop a food that stretches the stomach wall by taking advantage of the gelling ability of ionic polysaccharides.

I hypothesized that carbonated beverages containing ionic polysaccharides would foam and gel in the stomach to form a bubble-containing gel that would have an excellent stomach wall stretching effect and superior ingestibility, persistence, and convenience.

A gastric digestion simulator (GDS) that was recently developed to test gastric digestion *in vitro* makes it possible to directly observe the digestion process in real time. It can reproduce the physical conditions created by peristalsis *in vitro*. No other device possesses these features (Kozu *et al.*, 2014). MRI, which has been used in clinical trials, imposes a low physical burden on patients, and MRI scans can be quantitatively analyzed by integrating cross-sectional areas (Kozu *et al.*, 2010 & 2014). It appeared that MRI could be used to evaluate newly developed beverages.

Based on the above, the objective of this study was to develop a beverage that forms a bubble-containing gel in the stomach and produces a feeling of satiety, and to identify the factors that affect the gel's expandability and persistence.

1.7.2 Organization of this thesis

This study is an approach to appetite with beverages that can be consumed easily and continually. This thesis is divided into the following chapters.

Chapter 2 describes the part of this study in which a beverage that forms a bubble-containing gel when added to artificial gastric juice was developed. A carbonated beverage containing an ionic polysaccharide that a gel in the stomach and can be used in food should be useful as a means of stretching the stomach wall. An attempt was made to identify the optimal ionic polysaccharide and its concentration based on the magnitude of gel expansion and low viscosity. Then, clinical trials conducted using MRI and sensory evaluation were performed to determine whether the beverage developed actually forms a bubble-containing gel in the stomach and stretches the stomach wall sufficiently to induce satiety.

Chapter 3 describes the part of this study in which the factors that affect the gel volume and the retention capacity of the bubble-containing gel were investigated. In an experiment whose aim was to systematically evaluate the effects of beverage components and their

concentration, it was decided to use a continuous-type GDS (c-GDS), which has a mechanism for supplying artificial gastric juice with a pump and a mechanism for discharging gastric contents. The c-GDS conditions can be changed depending on the food to be evaluated. The c-GDS is an *in vitro* test device that simulates the chemical and physical environment of the human stomach, and it can be used to assess many factors that affect the volume of a bubble-containing gel in the stomach and their persistence. A systematic experiment identified some of the factors that affect the volume and the retention capacity of the bubble-containing gel. First, c-GDS test conditions were designed to evaluate the gastric digestion behavior of the bubble-containing gel, then beverage samples containing different pectin raw materials and citric acid concentrations were evaluated.

Chapter 4 summarizes the results described in Chapters 2 and 3 and discusses future research challenges and perspectives.

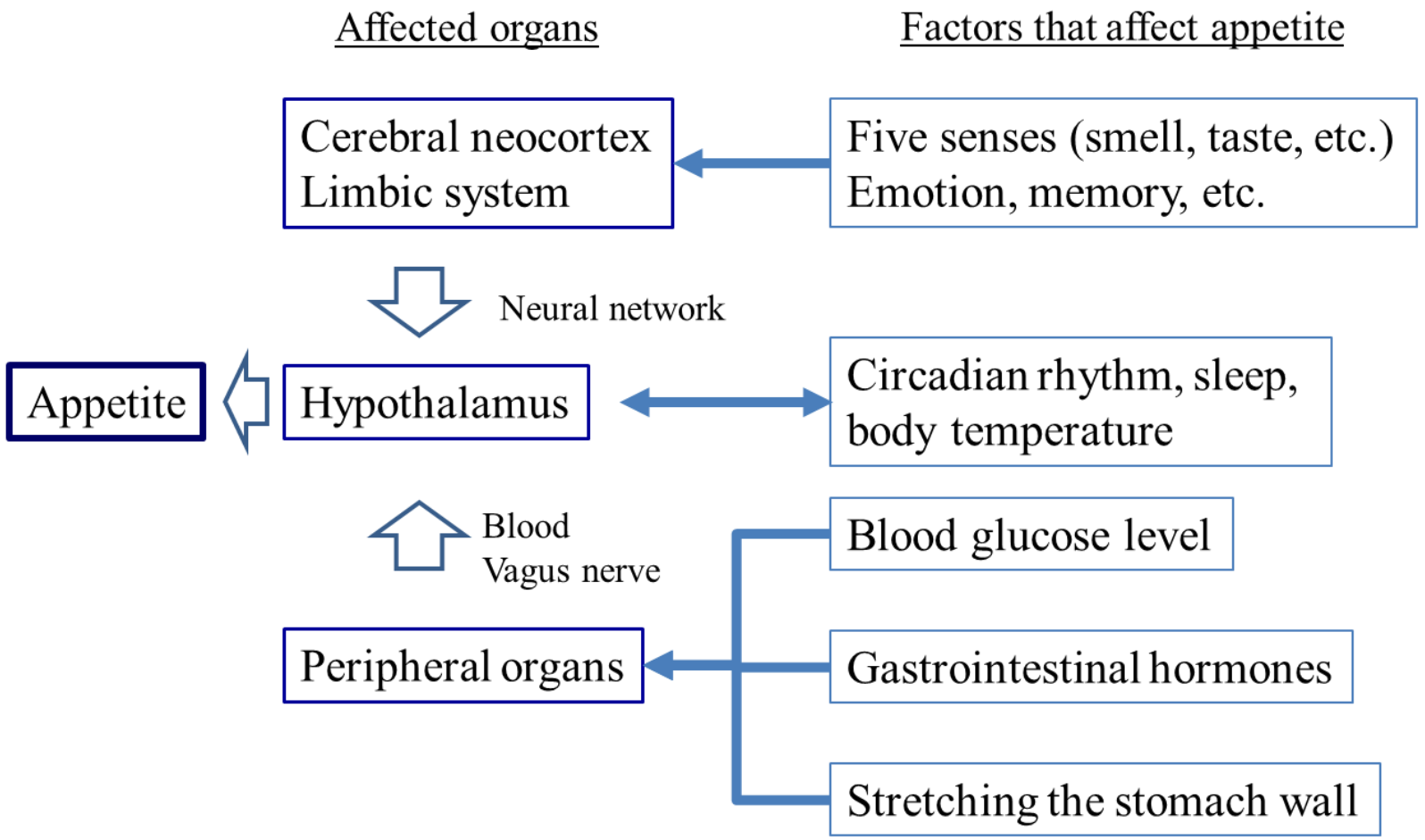

Fig. 1.1 Factors that affect appetite

Table 1.1 Beverages investigated to induce gastric distention

Name	Main ingredient	Advantage	Disadvantage	Reference
Carbonated beverage	Carbon dioxide gas	Ingestibility	Low persistence	Wakisaka *et al* ., 2012
High-viscosity beverage	Locust bean gum	Persistence	Low ingestibility	Marciani *et al* ., 2001
Intragastric-gelating beverage	Sodium alginate	Persistence	Low ingestibility	Hoad *et al* ., 2004
Bubble-containing beverage	Xanthan gum	Persistence	Low convenience	Murray *et al* ., 2015

Table 1.2 Pharmaceutical formulations for the treatment of heartburn and acid reflux

Polysaccharide (gelling agent)	Polysaccharide (other)	Bubble-forming agent	Reference
Sodium alginate	-	Sodium hydrogen carbonate	Malmud *et al* ., 1979
LM pectin	-	Sodium hydrogen carbonate	Havelund *et al* ., 1997
Sodium alginate	Xanthan gum	Sodium hydrogen carbonate	Brooks *et al* ., 1994
Sodium alginate	Guar gum	Sodium hydrogen carbonate	Gayst *et al* ., 1982
Sodium alginate	Locust bean gum	Sodium hydrogen carbonate	Dettmar *et al* ., 2003
Sodium alginate	Carrageenan	Sodium hydrogen carbonate	Chavkin *et al.* , 1986

CHAPTER 2. DEVELOPMENT OF A BEVERAGE THAT FORMS A BUBBLE-CONTAINING GEL IN THE STOMACH

2.1. Introduction

As stated in Chapter 1, since obesity leads to lifestyle-related diseases, it is important to prevent it. “Appetite control” is considered important. The methods of suppressing appetite with beverages that have been reported in the past to act by stretching the stomach wall have used beverages that are highly viscous, have poor ingestibility, and low persistence of the appetite suppressing effect, are prepared as needed, and are not very convenient. However, if a beverage that has excellent ingestibility, persistence, and convenience and stretches the stomach wall could be developed, it could be used to assist in obesity prevention. I therefore considered applying the gastric gelling preparation technique used in the pharmaceutical industry as a way to achieve ingestibility, persistence, and convenience. The pharmaceutical formulations for the treatment of heartburn and acid reflux include ionic polysaccharides that can also be used in food. By applying the gastric gelling preparation technique to food, it might be possible to develop a food that stretches the stomach wall.

It was thought that carbonated beverages containing ionic polysaccharides would gel as they foamed in the stomach to form a bubble-containing gel, and such beverages were expected to have an excellent stomach-wall-stretching effect as well as superior ingestibility, persistence, and convenience. Since before coming into contact with gastric juice ionic polysaccharides do not gel and have low viscosity, they have excellent ingestibility, the same as ordinary carbonated beverages. When ingested, the ionic polysaccharide gels in the stomach and forms a voluminous bubble-containing gel that contains carbon dioxide bubbles. The bubbles contribute to stretching the stomach wall and increasing the gastric retention and improving persistence. Moreover, if the beverage could be made available in bottles, such as cans or PET bottles or glass bottles, it would not be necessary to prepare it just before consumption and would be excellent in terms of convenience. No beverages having such characteristics have been reported in past studies.

To achieve such a beverage, it would be necessary to design it on the basis of *in vitro* tests and validate it in clinical trials. A variety of ionic polysaccharides are available, and it would be necessary to identify the optimal type and concentration of ionic polysaccharide.

The purpose of the part of this study described in this chapter was to develop a beverage that forms a bubble-containing gel in artificial gastric juice, to determine whether the beverage forms a bubble-containing gel in the human stomach and stretches the stomach, and to determine whether the beverage induces satiety by means of a clinical trial.

2.2 Materials and Methods

2.2.1 Materials

The Japan Pharmacopoeia 1st fluid for the disintegration test, pH 1.2, was purchased from Kanto Chemical Co., Ltd. Although various artificial gastric juice formulations are available, this formulation was selected because it has the simplest composition. The sodium alginate, carrageenan, xanthan gum, low-methoxyl pectin (LM pectin), high-methoxyl pectin (HM pectin), deacyl-type gellan gum, citric acid monohydrate, and sodium benzoate used in this study were food-additive-grade ingredients.

2.2.2 Preparation of the test beverage

Ionic polysaccharides were diluted with purified water or carbonated water (Table 2.1) and immediately used for the *in vitro* gelation tests. The citric acid and polysaccharide concentrations were set to an appropriate viscosity as a beverage, and their concentrations were also set to non-gelling and a proper buffering capacity. The sodium benzoate concentration was the same as commonly used in beverages.

2.2.3 *In vitro* gelation test

A 100 mL volume of test beverage cooled to 5°C or below was added to a 100 mL volume of artificial gastric juice (37°C). The state of the solution was evaluated visually, and the following formula was used to calculate the expansion rate of the gel:

$$\text{Expansion rate [\%]} = [(A-C)/B] \times 100 \quad (2.1)$$

A: Total volume [mL]
B: Test beverage volume [mL]
C: Artificial gastric juice volume [mL]

An automatic micro viscometer (AMVn, Anton Paar Co., Ltd., Graz, Austria) was used to measure viscosity 20°C at the time of preparation.

2.2.4 Clinical trial of a single consumption

In order to investigate whether the bubble-containing gel formation and expansion phenomena occur in the human stomach as well as *in vitro*, a clinical trial of a single consumption of the test beverage was conducted.

2.2.4.1 Participants

This study was conducted after receiving the approval of the Shiba Palace Clinic Ethics Review Committee on 28th January, 2016. In compliance with the spirit of the Declaration of Helsinki, the study was conducted in conformity with ethical guidelines (Ministry of Education, Culture, Sports, Science and Technology, Japan, and Ministry of Health, Labor and Welfare, Japan) for medical research on human beings. After properly explaining the contents of this study to the participants, written consent was obtained from each subject prior to participation in this study. Healthy adults from 20 to 64 years of age were eligible. Those who reported feeling slightly hungry or feeling hungry, just before consuming the test meal were selected as subjects. The target number of participants was 6. Participants with conditions that might affect the test results, i.e., with a history of a gastrointestinal disorder, possibly taking drugs, etc., were excluded.

2.2.4.2 Test meal and control meal

The carbonated beverage containing LM pectin (Kobara Support® apple flavor) was used as the test meal, and water (Livita® natural water) was used as the control meal. Kobara Support® contains pectin, acidulant, sodium benzoate, contains high-fructose corn syrup, indigestible dextrin, flavor, sweetener (acesulfame potassium, sucralose). It is a carbonated beverage containing 6.5 g of carbohydrate and 4.7 g of dietary fiber (4.2 g of indigestible dextrin, 0.5 g of pectin).

2.2.4.3 Clinical trial method

This study was a randomized, open-label, crossover (2 meals, 2 days) trial and was conducted in February 2016. The test meal and the control meal were consumed at least 6 days apart.

An MRI examination was performed immediately before ingesting the test meal or the control meal and at 10, 30, and 60 minutes after consuming it. MRI transverse photographs of the stomach were acquired at a slice thickness of about 3 mm to 5 mm during a breath-

hold. The MRI measurement conditions were optimized and set by making MRI measurements of a solution in which an acidic solution and the test meal or control meal had been mixed in advance. MRI of the same model was used during the test period.

A satiety questionnaire was administered 10, 30, and 60 minutes after consuming the test meal or the control meal.

2.2.4.4 Evaluation method

The primary endpoint was the non-formation/formation of a bubble-containing gel in the stomach following consumption of the test beverage. The image with the maximum cross-sectional area of the stomach was identified from the imaging data obtained 10 minutes after consuming the test meal. An example of an MRI photograph is shown in Fig. 2.1. The investigator judged whether a bubble-containing gel was visible in the photograph. The secondary endpoints were: 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 intragastric liquid volume and bubble-containing gel volume, and the responses to the satiety questionnaire. The bubble-containing gel area, the liquid area, and the gas area in all the photographed MRI images were calculated using the photograph analysis software (OsiriX; medical photograph analysis application). The internal volume of the stomach was calculated by the integration method. The satiety questionnaire responses were: “hungrier than before drinking,” “same as before drinking,” “slightly satisfied,” “somewhat satisfied,” or “satisfied.”

2.2.4.5 Statistical analysis

A statistical analysis was carried out in regard to the presence/absence of the bubble-containing gel in the stomach and responses to the satiety feeling questionnaire. Fundamental statistics were calculated for each measured value in regard to the internal volume of the stomach and the changes from immediately before consumption. The one-sample *t*-test was used for comparison with just before consumption, and the two-sample *t*-test was used for comparison with the control meal. SAS Ver. 9.1.3 software was used to perform the statistical analysis. The significance level was set at 5% (two-sided).

2.3 Results and Discussion

2.3.1 Formulation of the carbonated beverage that forms a bubble-containing gel upon contact with artificial gastric juice

Non-carbonated and carbonated aqueous solutions containing each of six representative ionic polysaccharides were prepared, and their ability to form a bubble-containing gel when mixed with artificial gastric juice was evaluated. When the carbonated aqueous solution of LM pectin, gellan gum, or sodium alginate was mixed with artificial gastric juice, a bubble-containing gel was formed (Table 2.1). Gel formation when an aqueous solution of LM pectin was added to gastric juice was reported previously (Itoh *et al.,* 2006; Hoad *et al.,* 2004). It was demonstrated for the first time that carbonated beverages containing these polysaccharides expand to form a bubble-containing gel in the present study. The gel formation was attributable to vaporization of the carbon dioxide gas when the solution was added to the artificial gastric juice. The solutions of HM pectin (in which more than half of the carboxyl groups were methoxylated), xanthan gum (which is small acidic sugar ratio), and carrageenan (which has sulfate ionic group) did not form a gel when mixed with artificial gastric juice. Thus, the ratio of carboxyl groups was concluded to be important for pH-responsive gel formation to occur. As an example the photograph taken after mixing the test beverage with the artificial gastric juice is shown in Fig. 2.2.

Since all three polysaccharides enabled the carbonated solutions to form a bubble-containing gel upon being mixed with gastric juice, the effect of the polysaccharide concentration on the expansion rate and viscosity at the time of preparation was assessed. The results revealed that LM pectin and gellan gum expanded markedly even at a low concentration of <0.1% (w/v) (Fig. 2.3a), but that addition of alginic acid to a concentration of 0.3% (w/v) did not induce gel formation, and that the expansion rate of alginic acid at the concentration at which gel formation was induced was low (Fig. 2.3a). The gelling concentrations were almost consistent with the conditions used in previous studies (Itoh *et al.,* 2006; Hoad *et al.,* 2004). The results of the present study showed for the first time that the expansion rate of the bubble-containing gel increased with their polysaccharide concentration. When the solutions were prepared at the same concentration, the solution containing gellan gum had a higher viscosity than the solution containing LM pectin (Fig. 2.3b), and thus gellan gum was unsuitable as the polysaccharide ingredient of a gel-forming beverage in the stomach from the standpoint of ingestibility. LM pectin was selected as the optimal polysaccharide for formulation of the test beverage in this study. The concentration

of LM pectin was set at 0.5%, because that concentration provided a moderate beverage viscosity and the maximum expansion rate of the bubble-containing gel in the stomach.

2.3.2 Clinical trial of a single consumption

The trial was carried out as planned in all six registered subjects. The data from all six subjects were included in the analysis. The subjects consisted of 2 males and 4 females. Their average age was 48.0 years (26-57 years), and their average BMI was 21.67 (18.9-24.8).

No formation of a bubble-containing gel was observed in any of the subjects following consumption of control meal (water), but a bubble-containing gel was observed in all of the subjects following consumption of the test meal (the carbonated beverage containing LM pectin). The formation of a bubble-containing gel in the stomach by a carbonated beverage was assessed by MRI for the first time.

The mean internal gastric volumes immediately before and 10 minutes after consumption are shown in Fig. 2.4 (the gas volume plus bubble-containing gel volume plus the liquid volume). The internal gastric volume had increased by 407 ± 211 mL (mean ± standard deviation) ($p = 0.005$) at 10 minutes after consumption of the carbonated beverage containing LM pectin, and it had increased by 190 ± 116 mL ($p = 0.010$) at 10 minutes following the consumption of water. Although the difference in the increase was 217 mL, it did not reach statistical significance ($p = 0.052$). No significant differences in volume were found at 30 minutes and 60 minutes after water consumption in comparison with volume recorded before consumption. A comparison of the results obtained with the test meal and showed that the total volume of the gel and liquid had increased by 182 mL at 10 minutes after consumption, and the difference was statistically significant ($p = 0.043$).

The same six subjects were evaluated for their feeling of satiety at 10 minutes after consumption. Their feeling of satiety tended to be greater after consumption of the carbonated beverage containing LM pectin than after consuming water (Fig. 2.5). The results of the satiety questionnaire showed that all of the subjects responded “slightly satisfied” or more at every evaluation point after consumption of the carbonated beverage containing LM pectin. At 10 minutes after consumption, 83% of the subjects who consumed the carbonated beverage containing LM pectin responded “somewhat satisfied”, as opposed to 33% after consuming water. The carbonated beverage containing LM pectin induced a higher degree of satiety than water did. None of the subjects said they were as hungry as they were before consuming the test beverage, and thus satiety had been induced in all of

the subjects. Therefore, it was concluded that the carbonated beverage containing LM pectin had the ability to induce satiety. This was the first study to demonstrate that a feeling of satiety was induced by the bubble-containing gel forming beverage in the stomach. However, induction of satiety by gel-forming beverages not containing carbonate has been reported previously (Hoad *et al.,* 2004). The carbonated beverage containing LM pectin may have produced a greater degree of gastric distension than water did.

Because the sample used for sensory evaluation contains fructose, high-fructose corn syrup, indigestible dextrin, flavor, and sweetener, the possibility that they affected the results of the sensory evaluation cannot be ruled out. Indigestible dextrin has been reported to be decomposed by bacteria in the large intestine to yield short-chain fatty acids, which induce satiety (Hobden *et al.,* 2015), and carbonated water is also known to induce satiety (Wakisaka *et al.,* 2012). In order to examine whether the bubble-containing gel affects satiety, it would be necessary to evaluate beverages that differed only according to whether they contained pectin. There is a possibility that the high-fructose corn syrup and indigestible dextrin affected satiety. Since a bulky diet has been found to induce a greater feeling of satiety (Melnikov *et al*., 2014; Peters *et al*., 2015), I concluded that gastric distension may have played a principal role in the induction of satiety in this study.

Beverages containing alginic acid or pectin have been reported to be effective in inducing satiety (Peters *et al.,* 2011; Wanders *et al.,* 2014), and several reports have indicated that the satiety following consumption of such beverages is attributable to gelation of the polysaccharides contained in them. Carbonated beverages containing LM pectin form a large gel in the stomach that produce greater distension of the stomach wall, which induces satiety.

Based on the above findings, it was concluded that consumption of the beverage developed would induce satiety, and thus the beverage was considered to be useful for reducing the risk of lifestyle diseases.

2.4 Conclusions

It is important to control appetite and to provide an easy method to control it in order to prevent obesity. In this chapter, a beverage that forms a bubble-containing gel in artificial gastric juice was developed. In addition, it was verified that the beverage forms a bubble-containing gel in the stomach and that it induced satiety in a clinical trial. The results reported in this chapter are as follows:

1）A beverage that forms a bubble-containing gel in the artificial gastric juice was developed.

2）It was demonstrated by MRI that the beverage forms a bubble-containing gel in the stomach and distends the stomach.

3）The results of a satiety questionnaire showed that the beverage induces satiety.

It was demonstrated that when mixed with artificial gastric juice carbonated beverages containing LM pectin form a gel and expand. The MRI study was performed to assess the formation of a bubble-containing gel in the stomach when the beverage was consumed. The results of a satiety questionnaire showed that the beverage induces satiety.

Table 2.1 Composition and characteristics of each test beverage

Ingredients [w/v%]												
Polysaccharide;	κ-Carrageenan		Xanthan gum		High-methoxyl pectin		Low-methoxyl pectin		Sodium alginate		Gellan gum (Deacyl type)	
	0.05	0.05	0.05	0.05	0.50	0.50	0.50	0.50	0.50	0.50	0.05	0.05
Citric acid monohydrate	0.05	0.05	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.05	0.05
Sodium benzoate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Aqueous solution	Non-carbonated	Carbonated	Non-carbonated	Carbonated	Non-carbonated	Carbonated	Non-carbonated	Carbonated	Non-carbonated	Carbonated	Non-carbonated	Carbonated
Properties after mixing with artificial gastric juice	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Gel	Expanded gel	Gel	Expanded gel	Gel	Expanded gel

The pH of the test beverage was adjusted with 1 mol/L of aqueous hydrochloric acid solution or 1 mol/L of aqueous sodium hydroxide solution. Because the test beverage containing gellan gum gelled at pH 4, and it was adjusted to pH 5.

A 100 mL volume of test beverage cooled to 5°C or below was added to a 100 mL volume of artificial gastric juice at 37°C. The results were observed after incubation at 37°C for 10 minutes.

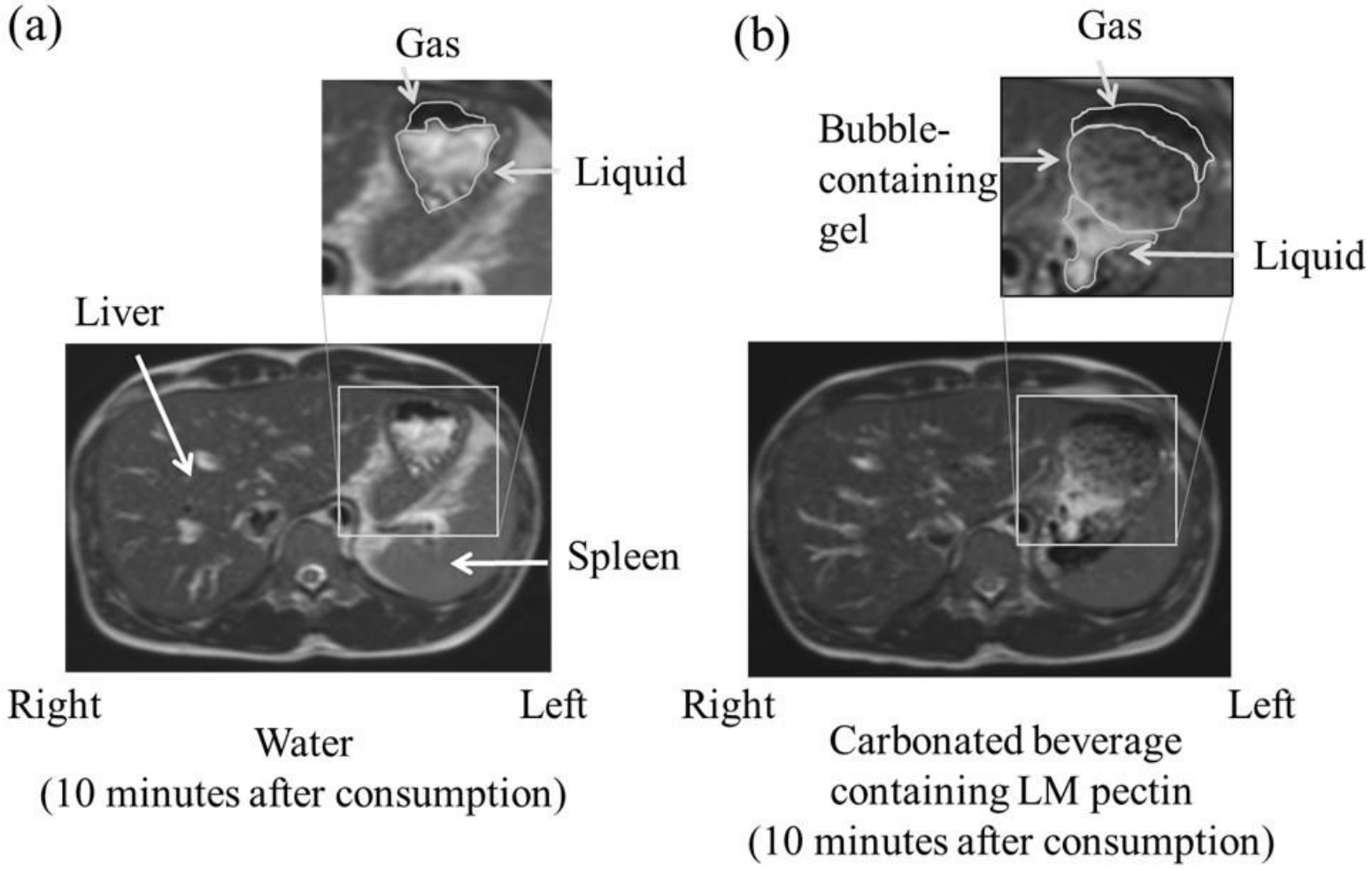

Fig. 2.1 Clinical trial of a single consumption in which MRI was performed

(a) Control meal(water) **(b)** Test meal (carbonated beverage containing LM pectin).

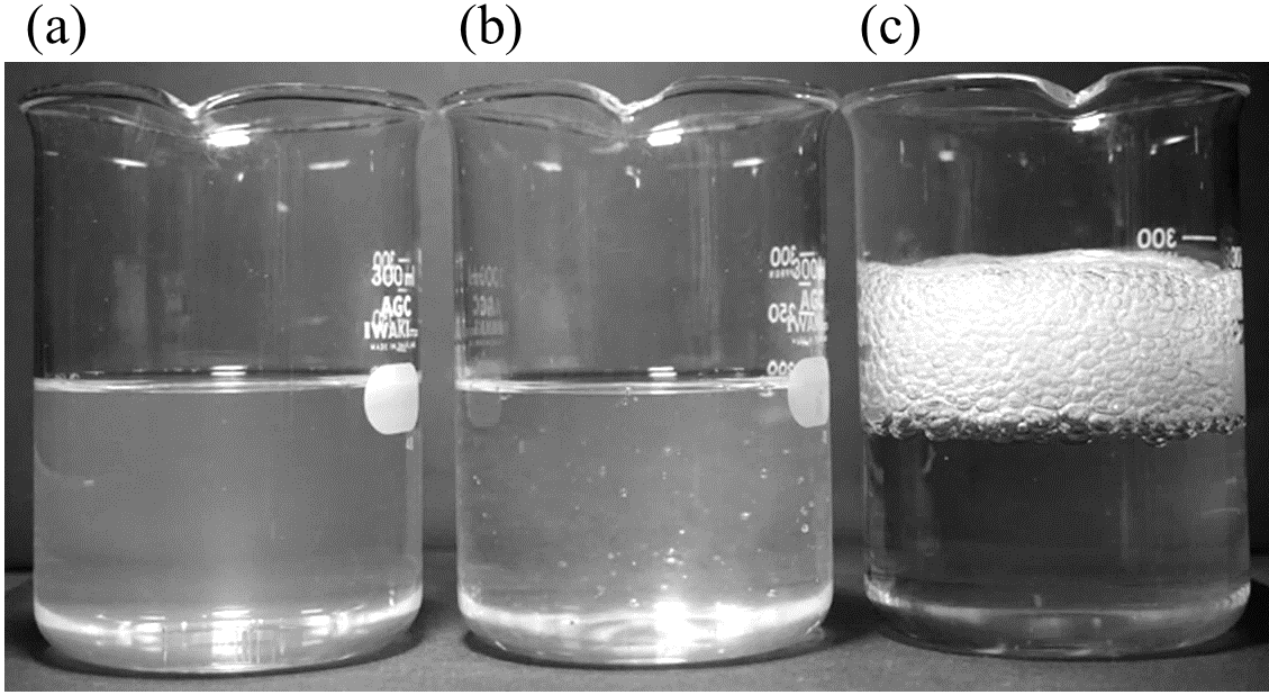

Fig. 2.2 The photograph taken after mixing a 100 mL volume of the test beverage with a 100 mL volume of artificial gastric juice **(a)** Carbonated water containing HM pectin, **(b)** Water containing LM pectin, **(c)** Carbonated water containing LM pectin
The polysaccharide concentration of all test beverages was 0.5%.

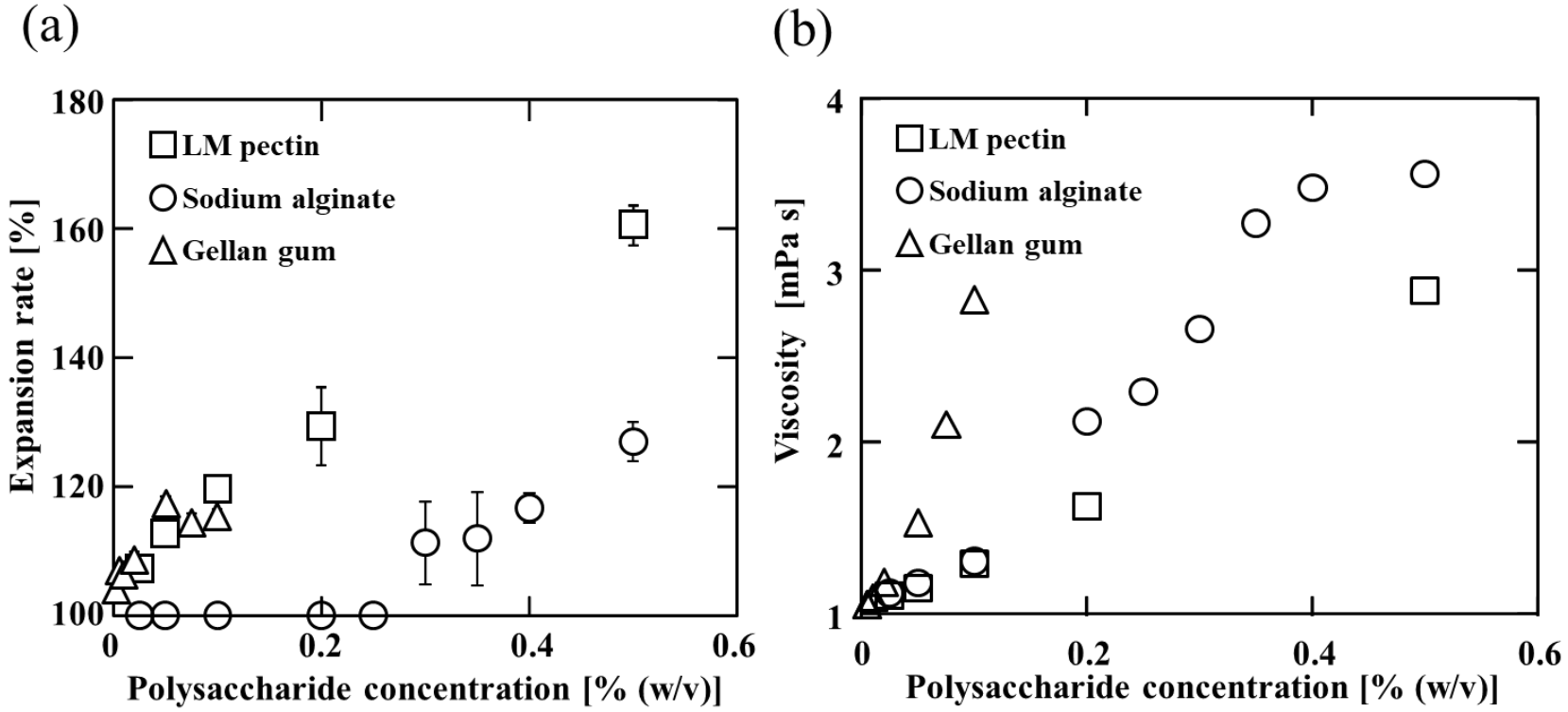

Fig. 2.3 Effect of polysaccharide concentration on the expansion rate of the gel and viscosity of the test beverage

(a) Effect of polysaccharide concentration on the expansion rate after a 100 mL volume of each test beverage was mixed with a 100 mL volume of artificial gastric juice.

(b) Effect of polysaccharide concentration on test beverage viscosity at the time of preparation.

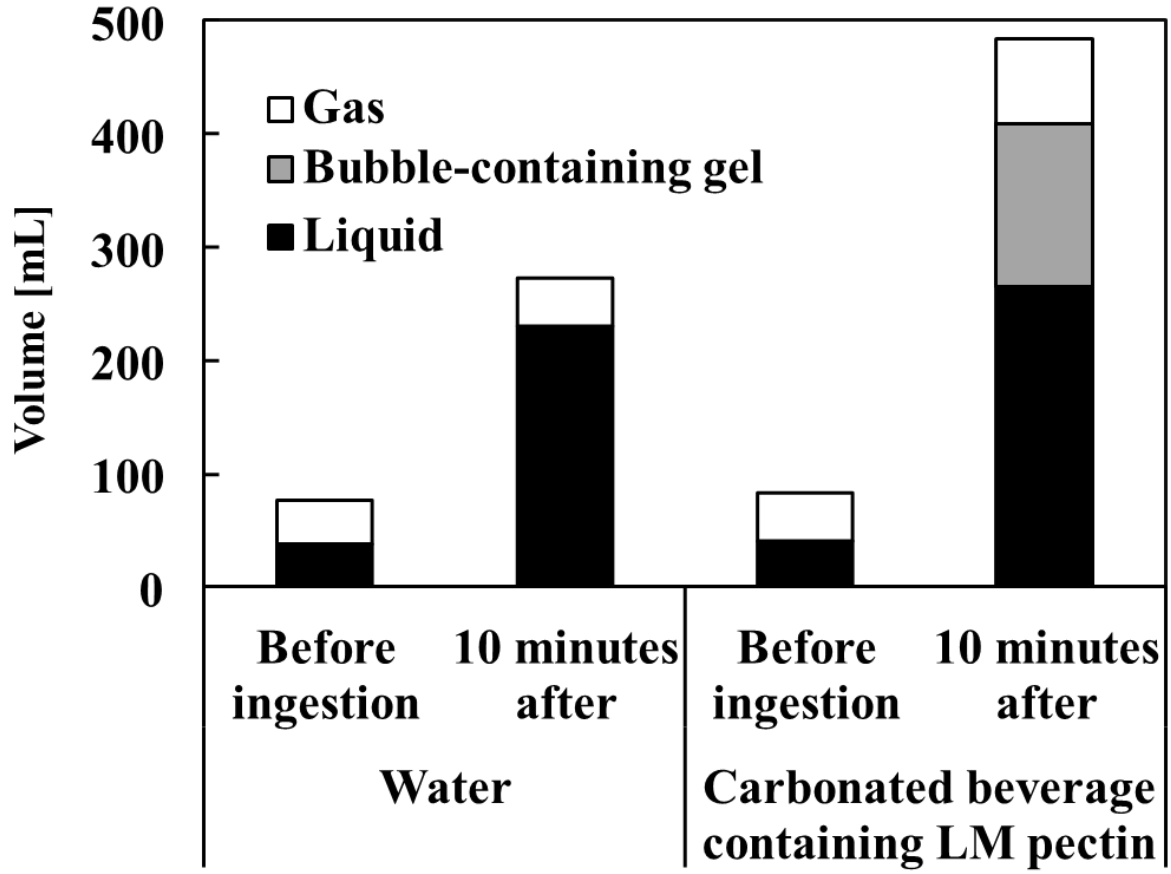

Fig. 2.4 Internal gastric volumes measured by MRI (n = 6)

Mean intragastric volumes immediately before consumption of the beverage and 10 minutes after consumption of the beverage (gas volume, bubble-containing gel volume, and liquid volume) are shown.

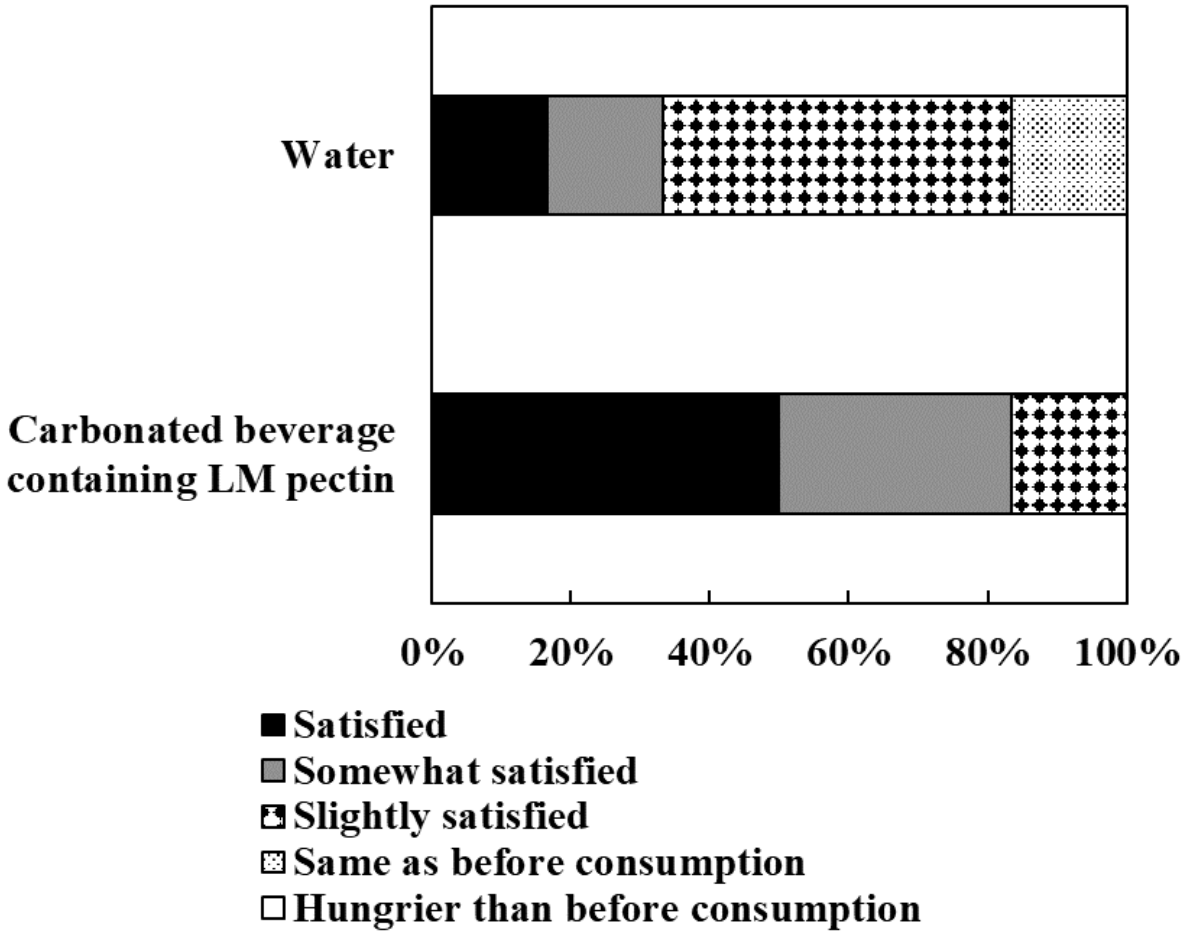

Fig. 2.5 Satiety questionnaire (n = 6)

The satiety questionnaire was administered 10 minutes after consuming each beverage.

CHAPTER 3. EVALUATION OF FACTORS AFFECTING THE VOLUME AND VOLUME-RETENTION CAPACITY OF A BUBBLE-CONTAINING GEL

3.1 Introduction

In chapter 2, the development of an LM pectin-containing carbonated beverage that expands and forms a bubble-containing gel in the stomach as a means of preventing obesity was described. It was demonstrated that carbonated beverages containing LM pectin formed a gel and expanded when mixed with artificial gastric juice, and an MRI study showed the formation of the bubble-containing gel in the stomach following consumption of a carbonated beverage containing LM pectin. The results of the satiety questionnaire revealed that the beverage induced a higher level of satiety than consumption of the same volume of water.

Since this beverage was just developed, it is not known how to optimize it to increase satiety. The stomach wall needs to be stretched for a longer time to increase satiety, and in order for the bubble-containing gel in the human stomach to stretch the stomach wall greatly and for a long time, it is important to generate a large bubble-containing gel and maintain its volume for a long time. Because it is difficult to evaluate large numbers of samples in clinical trials, an *in vitro* test that enables evaluation of numerous samples under conditions closer to the conditions in clinical trials is needed to achieve optimization.

The recently developed GDS is an *in vitro* human gastric digestion simulator that simulates the physical environment of the human stomach induced by peristaltic movements of the stomach wall. Since the side of the container simulating the pyloric part of the stomach is made of a transparent acrylic plate, the process of digestion of the stomach contents can be directly observed in real time (Kozu *et al.*, 2010 & 2014), a characteristic of the GDS not found in other human gastric digestion simulators. The GDS is a digestion simulator that was suitable for use in this study to evaluate the volume of the bubble-containing gel in the stomach over time. In addition, the continuous-type GDS (c-GDS) (Kozu *et al.*, 2017), which is equipped with a mechanism for supplying artificial gastric juice with a pump and a mechanism for discharging gastric contents, that can be used according to the food being evaluated. The c-GDS makes it possible to simulate various stomach conditions by controlling the volume of gastric juice secreted and the volume of stomach contents discharged into the intestine.

Various factors are expected to affect the size of the bubble-containing gel and its persistence. The basic components of the beverage whose development was described in

Chapter 2 are LM pectin and citric acid. LM pectin is mainly used as raw materials in the production of jellies and jams, and it is processed and prepared from natural products such as citrus fruits and apples by a variety of methods. Various types of LM pectin are commercially available to enable preparation of jellies and jams having different textures. Citric acid is used in various concentrations, depending on the flavor and pH of the beverage. The purpose of the parts of the study described in this chapter was to identify effects of the type of LM pectin raw material and the concentration of citric acid.

The purpose of this chapter is investigating the factors that affect the volume of the bubble-containing gel and its volume-retention capacity. To do so, c-GDS test conditions for the bubble-containing gel were designed based on clinical trial results for the gastric discharge rate of beverages, and beverage samples containing different pectin ingredients and citric acid concentrations were evaluated using the c-GDS test conditions.

3.2 Materials and Methods

3.2.1 Materials

As mentioned Chapter 2.2.1, Japan Pharmacopoeia 1st fluid for disintegration test, pH 1.2, was purchased from Kanto Chemical Co., Ltd. LM pectin (Unitec Foods Co., Ltd., Tokyo, Japan), citric acid monohydrate (Iwata Chemical Co., Ltd., Shizuoka, Japan) and sodium benzoate (DSP Gokyo Food & Chemical Co., Ltd., Osaka, Japan) were used. These are all food additive grades. The product names of the LM pectin used in this study were: pectin A, OF327C; pectin B, AYS407C; pectin C, OF445C; pectin D, OF463CSB; pectin E, OF805; and pectin F, LMSN325.

3.2.2 Preparation of the test beverage

The beverage that forms a bubble-containing gel in the stomach was prepared by diluting ionic polysaccharides with purified carbonated water (Table 3.1). Beverage samples were placed in aluminum cans and sterilized at 80°C for 20 minutes. They were used for the *in vitro* gastric digestion test. The sodium benzoate concentration was the same as commonly used in commercial carbonated beverages.

3.2.3 Measurements of the physical properties of the gel

Beverage samples not containing carbon dioxide were used to measure the physical properties of the gel. An 80 mL sample was placed in 40-mm dialysis tubing (fractionated molecular weight = 6,000-8,000 Spectra/Por 1 Membrane Spectrum Laboratories Inc.,

Rancho Dominguez, CA), dialyzed for 20 hours in 1600 mL of artificial gastric juice, and gelled. The gel samples were positioned horizontally, and their breaking stress and Young's modulus were measured with a 1-cm-diameter flat jig and a rheometer (FUDOH Rheometer RTC Rheotech, Ltd., Tokyo, Japan). The descending speed and the strain rate of the jig were set at 1 cm/min and 100%, respectively. The physical properties of the gel were calculated from the average of three measurements. The stress-strain curve shown in Fig. 3.1 is an example of the measurement of the physical properties of a gel.

3.2.4 *In vitro* gastric digestion experiment

3.2.4.1 Addition of the test beverage to artificial gastric juice

A 50 mL volume of artificial gastric juice (Ricci *et al.,* 1993), which is the volume of gastric juice in the human stomach in the fasting state, was poured into the c-GDS or an acrylic vessel (70 × 70 × 150 mm). A rubber tube having an inner diameter of 19 mm and length of 250 mm, corresponding to the inner diameter and length of the esophagus, was connected to a funnel having a diameter of 120 mm. This device was used to pour 185 mL of the test beverage cooled to 5°C or less into a c-GDS or an acrylic vessel over approximately 7 seconds at an inclination of about 45º.

3.2.4.2 Design of c-GDS test conditions for a bubble-containing gel

The test conditions of c-GDS were set with reference to test conditions described in the clinical literature that the discharging rate of the beverage was described (Sun *et al.*, 1988; Kararli, 1995; Marciani *et al*., 2001). The temperature was set at 37 °C, the initial gastric fluid volume was set at 50 mL, and the apparent gastric emptying rate was set at 1.47 mL/minute (Fig. 3.2), and the evaluation time was set at 120 minutes.

In order to approximate the conditions to the digestive behavior in the human stomach, the following two criteria were used to evaluate the validity of the evaluation system: the liquid temperature in the stomach model should rise to 35°C or more by 30 minutes after sample input, and gastric juice drainage should not have ended by 120 minutes.

Since gastric juice supply and excretion vary with food intake and elapsed time, two conditions (conditions A/B below) were set for gastric fluid supply and excretion, based on the gastric emptying rate reported in the clinical literature (Murray *et al*., 2015). The volume change of the bubble-containing gel and the liquid phase pH in the stomach model were evaluated in order to select appropriate conditions from the two conditions. The sample used was Sample A-1 shown in Table 3.1.

Condition A

- Gastric juice supply rate: 2.0 mL/min
  (maximum calculated from a reference (Marciani *et al*., 2001))
- Gastric juice excretion rate: 3.47 mL/min (2.0 mL/min + 1.47 mL/min)

Condition B

- Gastric juice supply rate: 0.5 mL/min
  (minimum value calculated from a reference (Marciani *et al*., 2001))
- Gastric juice excretion rate: 1.97 mL/min (0.5 mL/min + 1.47 mL/min)

3.2.4.3 Observation and evaluation of *in vitro* gastric digestion experiments

The static evaluation was performed by incubating the acrylic vessel for 90 minutes at 37°C. The generation frequency of the antral contraction wave (ACW) and c-GDS speed were set to 1.5 cycles/minute and 2.5 mm/second, respectively (Kozu *et al.*, 2010 & 2014). A c-GDS equipped with an artificial gastric juice supply and emptying system was used to simulate the effects of gastric juice secretion and emptying *in vivo* (Kozu *et al.*, 2017). The vessel of c-GDS was incubated at 37°C for 90 minutes.

The digestion process in the c-GDS or the acrylic vessel was evaluated by analyzing the course of changes in the volume of the bubble-containing gel. The mean gel volumes (n = 4) at 10, 30, 60, and 90 minutes after addition of the test beverage to the artificial gastric juice were calculated. The gel was photographed from the front of the c-GDS or the acrylic vessel. The volume of the gel at each time-point was calculated by using the "Image J version 1.3.4.67." image analysis software program and the following formula:

$$\text{Gel volume [mL]} = (A/B) \times 300 \text{ mL} \quad (3.1)$$

A: Projected cross section of the gel [cm^2]

B: Projected cross section of the 300-mL vessel [cm^2]

The gel volume retention rate was calculated using the following formula:

$$\text{Gel volume retention rate [\%]} = C/D \quad (3.2)$$

C: Gel volume at 90 minutes after addition [mL]

D: Gel volume at 10 minutes after addition [mL]

3.3 Results and Discussion

3.3.1 Design of c-GDS test conditions for a bubble-containing gel

The c-GDS test conditions were set to evaluate the gastric digestion behavior of the bubble-containing gel. The c-GDS measurement conditions (temperature, initial gastric juice volume, and apparent gastric emptying/evaluation time) were set based on a study that assessed the gastric emptying rate during drinking (Murray *et al*., 2015). Based on the gastric emptying rate reported in the literature (Murray *et al*., 2015), two conditions were set for gastric fluid supply and excretion.

The results of the evaluation of these two conditions are shown 3.3.1.1-3.3.1.4.

3.3.1.1 Evaluation of the gastric juice temperature

Fig. 3.3 shows the results of evaluating the gastric juice temperature of conditions A and B twice to assess reproducibility. Since all of the measurements showed that the temperature was 35 °C or more after 20 minutes and never exceeded 40°C, both conditions were considered appropriate.

3.3.1.2 Evaluation of the volume of a bubble-containing gel

The results of calculating the gel volume based on the c-GDS evaluation photographs (Fig. 3.4) are shown in Fig. 3.5. The results of performing the measurements twice under conditions A and B showed good reproducibility under both conditions. Although the digestion behaviors were similar until 90 minutes, after 120 minutes there was a difference of about 20% in the gel volume between conditions A and B.

3.3.1.3 Evaluation of the gastric juice pH

Based on the pH measurements in the stomach model (Fig. 3.6), after 120 minutes the pH was lower under condition A. It was considered that the difference in gastric juice supply and discharging affected the gastric juice pH.

3.3.1.4 Summary of the test results

Comparison of the results under the two conditions showed that there was the difference in the gel volume after 120 minutes. However, other digestion behaviors were similar. Since there are the difference in the gastric juice pH, the difference in the gel volume after 120 minutes may be due to the pH of gastric juice. Because the degree of dissociation of

carboxyl groups decrease as pH decreasing, the formed gel strength is known to be weak below pH 2 (Kawabata, 2015).

Since gastric pH returns to pre-feeding values in 2-3 hours (Asahi *et al*., 1986), based on the results shown in Fig. 3.6, condition A was concluded to be closer to conditions *in vivo* and was used in the subsequent evaluation.

3.3.2 Comparison of *in vitro* gastric digestion experiments

Two different tests, a static evaluation test and a dynamic evaluation test by c-GDS, were used to determine how the bubble-containing gel changed with time. The static evaluation test was performed using an acrylic vessel in order to evaluate the stability of the bubble-containing gel, and the results showed that the gel expansion was not transient and was sufficiently strong, and that the increased volume was stably maintained (Fig. 3.7a). The sample had expanded to an approximately 1.3-fold volume at 10 minutes after adding it to artificial gastric juice, and its volume was maintained at 75% even after 90 minutes (Fig. 3.8). The dynamic evaluation test was conducted using the c-GDS in order to evaluate the resistance of the gel to digestion in the human stomach in view of the effects of peristalsis. As the bubbles burst at the bottom end, the gel gradually shrank (Fig. 3.7b), but its volume was maintained at 40% even after 90 minutes despite the steady decrease in gel volume (Fig. 3.8).

The bubbles in part of the gel burst because of the direct effects of peristalsis, and because the bubbles disappeared, the gel gradually shrank from the outside. This digestion behavior was different from that of *tofu* (Kozu *et al.*, 2014), a typical solid food. In general, solid foods are chewed and broken down into small fragments. The fragments are then swallowed and reach the stomach, where they are digested into fine particles. By contrast, a bubble-containing gel form a single large mass and gradually shrink over time in the stomach. Because the c-GDS stomach model is an open system, even if the gel is compressed by the peristaltic movements, it is only pushed out upward. Since the intragastric volume of the gas part of stomach measured by MRI was small, the bubbles may readily be collapsed by peristaltic movements.

From these results, the dynamic evaluation test by c-GDS was used in the subsequent evaluation.

3.3.3 Evaluation of Factors Affecting the Volume and Volume-Retention Capacity of a Bubble-Containing Gel

3.3.3.1 Effect of the type of pectin on the course of changes in gel volume

The effect of the type of pectin on the course of changes in gel volume was evaluated. As shown in Fig. 3.9, at both 10 minutes and 90 minutes after bubble-containing gel formation there were differences in gel volume according to the type of pectin used (maximum difference in gel volume 22% at 10 minutes after gel formation; maximum difference in gel volume 27% at 90 minutes after gel formation). At 10 minutes, the gel volume of the formulation prepared with LM pectin E was smaller, and at 90 minutes it was also smaller than obtained with the formulation prepared with LM pectin B/C/D. These findings indicated that the gel volume at 10 minutes after gel formation predicted the gel volume at 90 minutes. Differences in gel volume and the digestive behavior of gel may be affected by differences in the characteristics (degree of esterification, degree of amidation, *etc.*) of the pectin raw material used. Since the parameters of the pectin raw material affect the physical properties of the gel, the parameters may affect gel volume.

Photographs of the digestive behavior of the beverage samples prepared using LM pectin B and E are shown in Fig. 3.10. When beverages that form a bubble-containing gel in the stomach were added to the c-GDS filled with artificial gastric juice, generation of a bubble-containing gel was observed. The sample prepared with LM pectin B formed a gel containing large bubbles, whereas the sample prepared with LM pectin E formed a gel containing fine bubbles. It appeared that the more a beverage forms a gel that entraps large bubbles, the larger the total volume of the bubble-containing gel becomes.

The test conditions that reduced the volume of the artificial gastric juice were used in this experiment. The photographs showed that the liquid level decreased as the volume of the artificial gastric juice decreased, and the gel volume was seen to decrease with time due to peristalsis. The samples prepared using LM pectin B shrank but retained their tubular shape, whereas the samples prepared using LM pectin E contracted into a sphere. It appeared that differences in the physical strength of the bubble-containing gel may affect the rate of contraction of the gel.

3.3.3.2 Effect of the citric acid concentration in the formulation on the course of changes in gel volume

The c-GDS was used to evaluate the effect of the citric acid concentration on the gel volume and its retention capacity. As shown in Fig. 3.11, there were differences in the gel volume at both 10 minutes and 90 minutes according to the differences in citric acid

concentration. The maximum difference in gel volume at 10 minutes after gel formation was 28%, and the difference in gel volume at 90 minutes after gel formation was 26%.

Photographs of the digestive behavior of Sample A-1 and Sample A-2 are shown in Fig. 3.12. The shape of the gel differed according to the citric acid concentration, even when the same type of pectin was used. The volume of the gel and size of the bubbles decreased when the citric acid concentration was high. Higher concentrations of buffering agents usually provide greater buffer capacity. The pH after the mixing of sample with artificial gastric juice increased because the buffering capacity was raised up by the addition of citric acid in the sample. In this case, the gelation proceeded not so well because of high pH, and then large air bubbles could not be trapped in the gel. It was concluded that the volume of bubbles trapped by the gel was affected by the difference in gelation of sample due to the differences in the pectin raw materials and the citric acid concentrations.

3.3.3.3 Identification method of factors that affect gel volume

A correlation analysis was performed in an attempt to identify factors that affect gel volume and gel volume retention capacity. Since the degree of esterification and the degree of amidation depend only on the type of pectin material, samples prepared under the same conditions (citrate concentration: 0.2%) were used. Formula 3.2 was used to calculate gel volume retention capacity from the ratio of gel volume at 10 minutes and at 90 minutes.

3.3.3.4 Factors that affect gel volume immediately after the reaction between the sample and gastric juice

Gel volume at the 10-minute evaluation was correlated with the degree of esterification (Fig. 3.13, correlation coefficient: R = -0.73). The fraction of protonated carboxyl group in LM pectin increase at low pH, and then hydrogen bonds between pectin as polysaccharide polymer are more likely form in low degree than high degree of esterification (Fraeye *et al.*, 2010). As a result, gelation is more likely to occur for LM pectin having low degree of esterification, and the volume of the sample increases. Large bubbles can be trapped in the gel, and the volume of the gel increases immediately after the reaction (Fig. 3.10 Sample B).

Conversely, when the degree of esterification is high, the gelation proceeded not so well because more carboxyl groups of pectin are methoxylated. Therefore, a smaller amount of bubbles was trapped, and as a result, the volume of the bubble-containing gel was smaller.

At 10 minutes after gelation, the correlation between gel volume and degree of amidation was weak (R = -0.16).

3.3.3.5 Factors that affect gel volume retention capacity

As shown in Fig. 3.14a, the correlation between gel volume retention capacity and degree of esterification was weak (R = -0.29). By contrast, as shown in Fig. 3.14bcd, there were strong correlations between gel volume retention capacity and the degree of amidation (R = 0.69), breaking strength (R = 0.65), and Young's modulus (R = 0.63). Because hydrogen bonds form between amide groups (Chan *et al.*, 2017), amidated pectin is known to form stronger gels at low pH (Lootens *et al.*, 2003). The coefficients (R) of the correlations between degree of amidation and both breaking stress (0.77) and Young's modulus (0.66) were relatively high (data are not shown). The degree of amidation can indirectly affect gel volume retention through breaking strength and Young's modulus. When the degree of amidation is high, cross-link between pectin polymers is more likely to develop. As a result, the strength of the gel increases and the retention capacity of the gel volume increase.

3.3.3.6 Summary of the analysis of factors

Even in the absence of calcium ions, LM pectin forms a gel at pH's below 3.5. The results of the experiments described in this chapter showed that formulation conditions with pectin having a low degree of esterification and with low citric acid concentrations increased the size of the bubble-containing gel volume immediately after reaction with gastric juice. It was also concluded that the higher degree of pectin amidation were responsible for the higher breaking strength and Young's modulus that were associated with higher gel volume retention capacity. Therefore, involvement of a higher proportion of the sample in gel formation is important to increase the initial volume of the bubble-containing gel, and the strength of the gel is considered important to maintaining the expanded gel volume.

Because pectin is a natural product, it has a complex steric structure and wide molecular weight distribution, and thus factors other than the degree of esterification and degree of amidation may affect expanded gel volume and volume retention capacity. In order to accurately evaluate the factors that affect gel volume and volume retention capacity, it will be necessary to use well characterized materials having different parameters, such as synthetic polymers.

Although the experiment in this chapter was an *in vitro* experiment, it was possible to identify factors that affect the volume of the bubble-containing gel and its retention capacity. This is the first research work that revealed the factors affecting the formation and gastric digestive behavior of a bubble-containing gel. Using a c-GDS made it possible to identify superior types of pectin in regard to bubble-containing gel formation. The results of these experiments will be valuable for developing more effective beverages to prevent obesity. Even for a non-gel forming beverage, it has been reported that the gastric emptying rate of beverage is slower when a stable foam is formed than when an unstable foam is formed, and the level of satiety increase with the degree of stability of the foam (Murray *et al.,* 2015). Longer volume retention of bubble-containing gel will slow the gastric emptying rate of a bubble-containing gel, so the satiety will be kept longer.

3.4 Conclusions

In order to optimize the formulation of beverages that form a bubble-containing gel in the stomach, it is important to know which factors affect the volume of the bubble-containing gel and its volume-retention capacity. To do so, c-GDS test conditions for the bubble-containing gel were designed, and samples containing different pectin ingredients and citric acid concentrations were evaluated by using the c-GDS test conditions.

The primary results reported in this chapter are as follows:

1）The c-GDS test conditions were designed so that they approximated the conditions reported in the results of clinical trials.

2）The results of using the c-GDS test conditions showed that the digestion behaviors of the bubble-containing gel varied with the citric acid concentration and the degree of esterification and degree of amidation of the pectin ingredients. A correlation analysis of the evaluation results showed that they affected the volume of the bubble-containing gel and its retention capacity.

In the part of the study described in this chapter, factors that affect the volume and retention capacity of the bubble-containing gel were identified by evaluating its digestion behavior with a c-GDS. The degree of esterification of pectin and the citric acid concentration were found to affect gel volume immediately after reaction with artificial

gastric juice. The degree of amidation of pectin and the physical properties of the gel (breaking stress and Young's modulus) were also found to affect the gel volume retention capacity. The degree of esterification and degree of amidation of pectin and the citric acid concentration in the formulation must be taken into consideration in order to maximize the volume of the gel and its volume retention capacity when the beverage is ingested in stomach.

This study was the first to evaluate factors that affect the formation and digestive behavior in the stomach of a bubble-containing gel. The findings are useful for developing a beverage that produces satiety by forming a bubble-containing gel in the stomach according to its composition.

Table 3.1 Composition of the beverage samples that form a bubble-containing gel in the stomach

Beverage sample name	Pectin				Constituent conc.*			Physical properties of gel without carbon dioxide	
	Type of pectin	Origin	Degree of esterification [%]	Degree of amidation [%]	Pectin [w/v%]	Citric acid monohydrate [w/v%]	Sodium benzoate [w/v%]	Breaking stress [kPa]	Young's modulus [kPa]
Sample A-1	A	Citrus fruit	34	14	0.50	0.20	0.05	5.3	16.6
Sample A-2	A	Citrus fruit	34	14	0.50	1.00	0.05	1.6	9.1
Sample B	B	Citrus fruit	27	20	0.50	0.20	0.05	11.4	23.2
Sample C	C	Citrus fruit	28	18	0.50	0.20	0.05	11.9	26.4
Sample D	D	Citrus fruit	28	10	0.50	0.20	0.05	3.6	16.3
Sample E	E	Apple	34	15	0.50	0.20	0.05	3.1	11.9
Sample F-1	F	Apple	29	18	0.50	0.20	0.05	6.1	20.4
Sample F-2	F	Apple	29	18	0.50	1.00	0.05	6.0	19.3

* The pH of each sample was adjusted to 4 with a 1 mol/L aqueous hydrochloric acid solution or 1 mol/L aqueous sodium hydroxide solution.

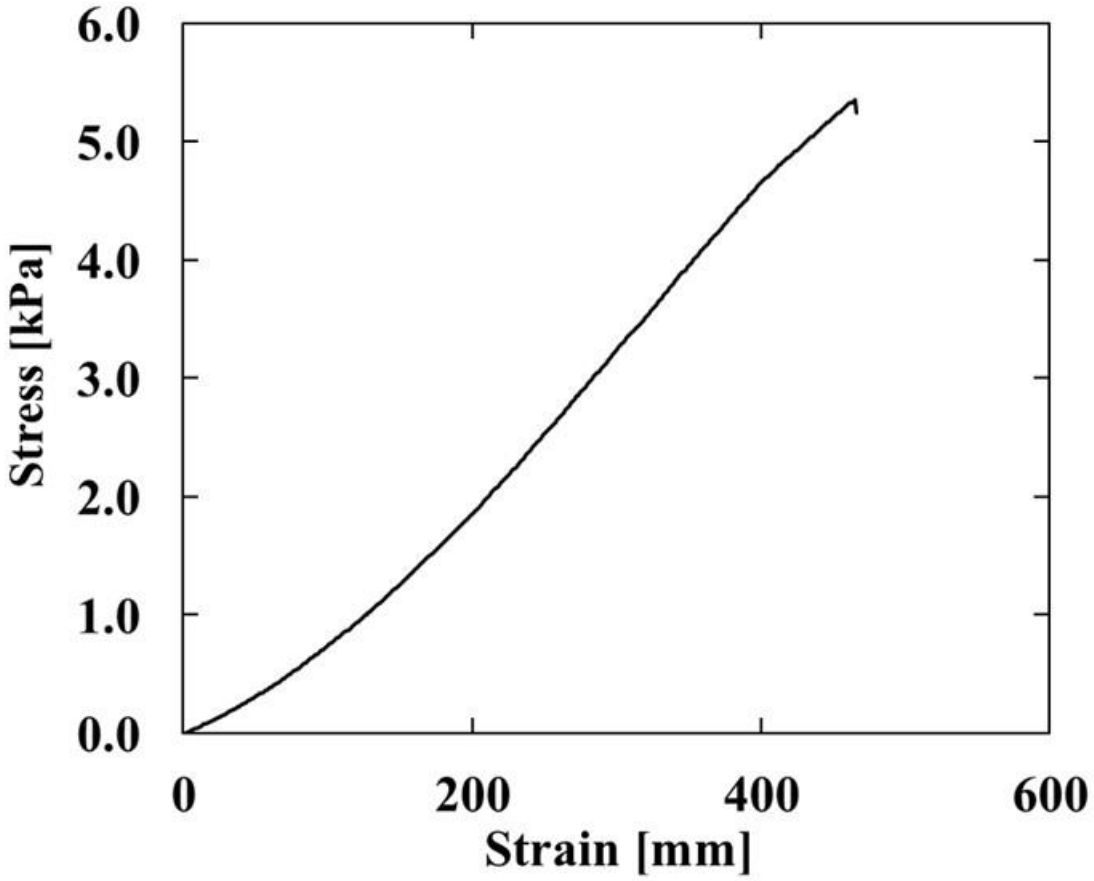

Fig. 3.1 The stress-strain curve of a gel (Sample A-1)

A gel without carbon dioxide gas was used to determine the physical properties shown in Table 3.1.

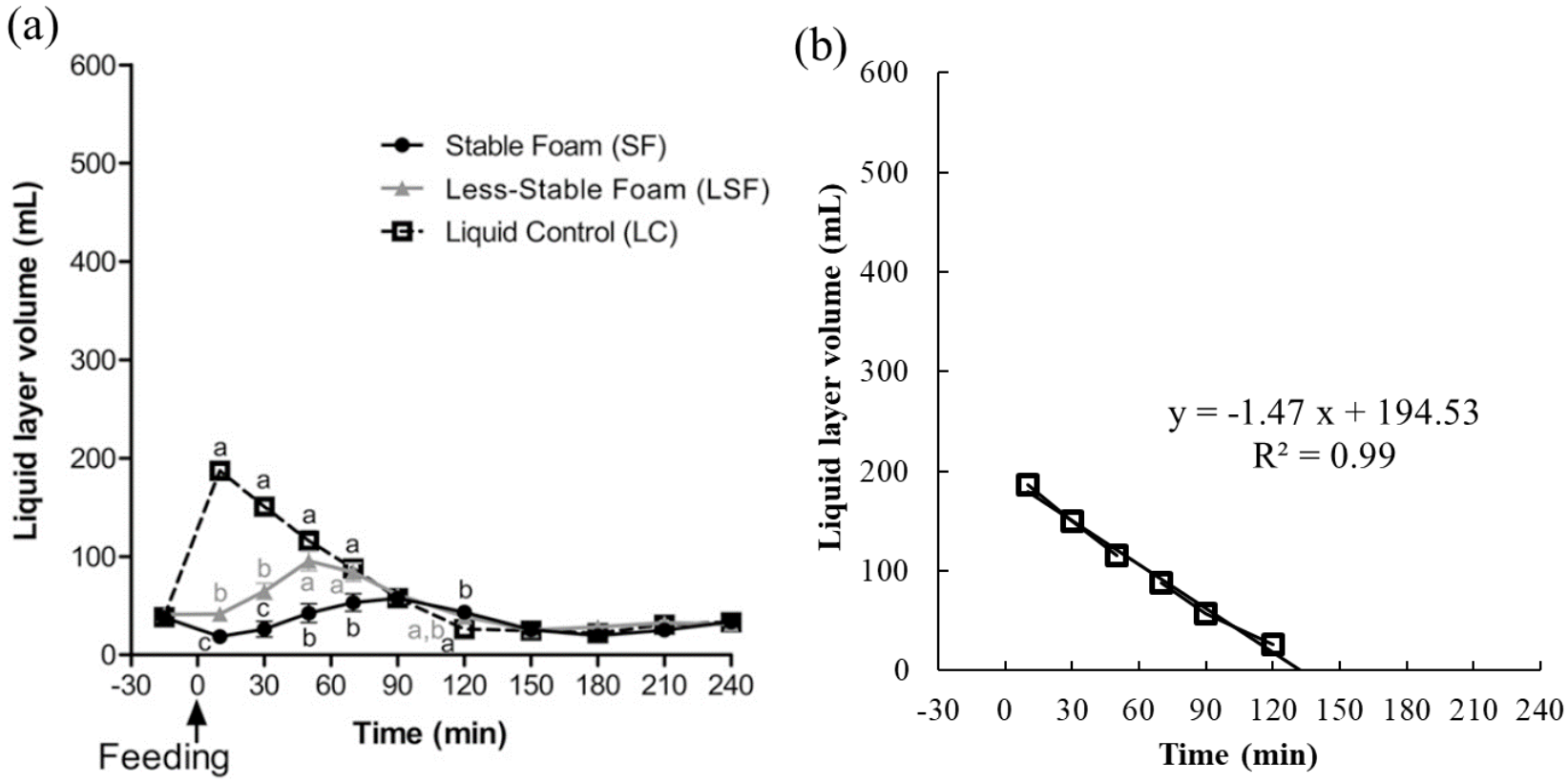

Fig. 3.2 Calculation of gastric emptying rates based on clinical test reports of the gastric emptying rates of aerated beverages

(a) Residual amount of contents in the human stomach when the beverages (Murray *et al*., 2015)

(b) Approximation of gastric emptying rate calculated from the volume reduction behavior of a “Liquid Control” sample

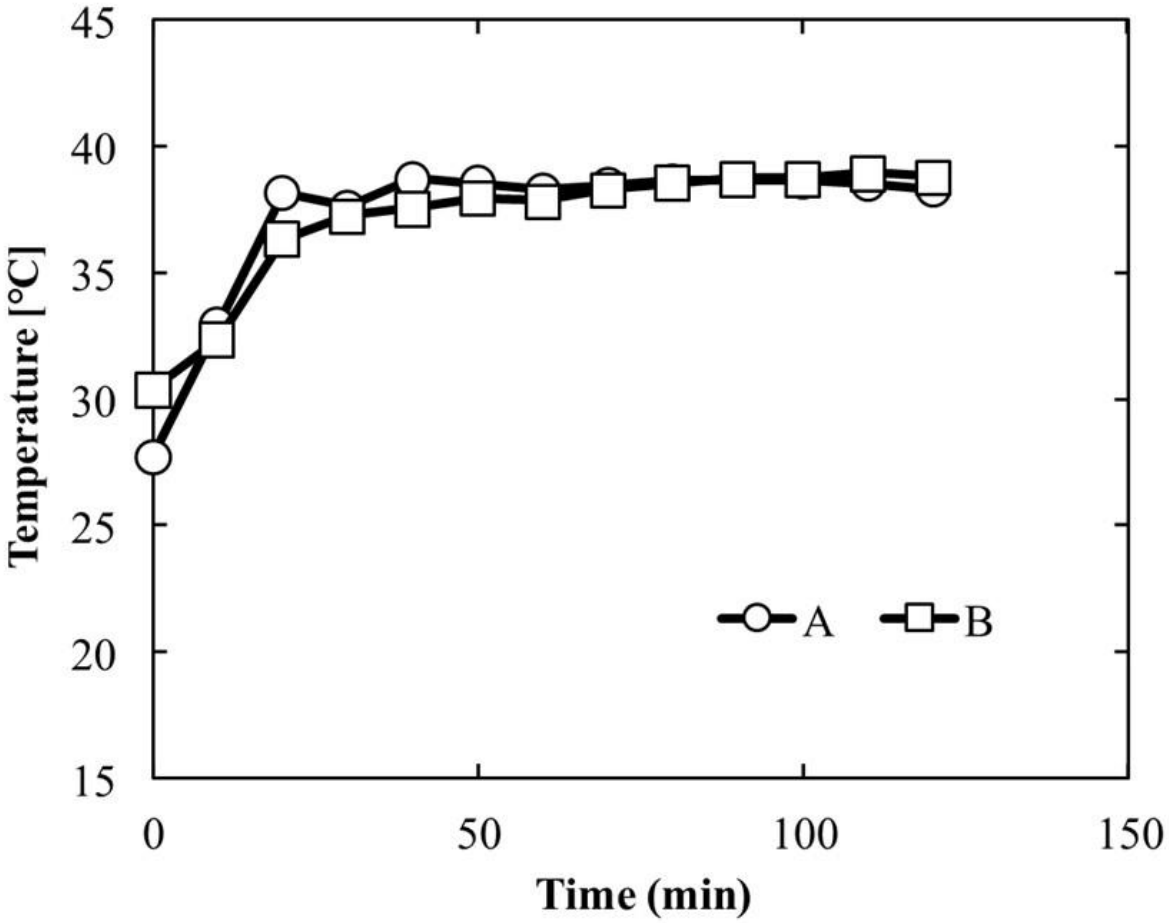

Fig. 3.3 Temperature in the stomach model under condition A and condition B

The temperature in the stomach model after introduction of Sample A-1 in Table 3.1 was measured under condition A and condition B shown in Chapter 3.2.4.2.

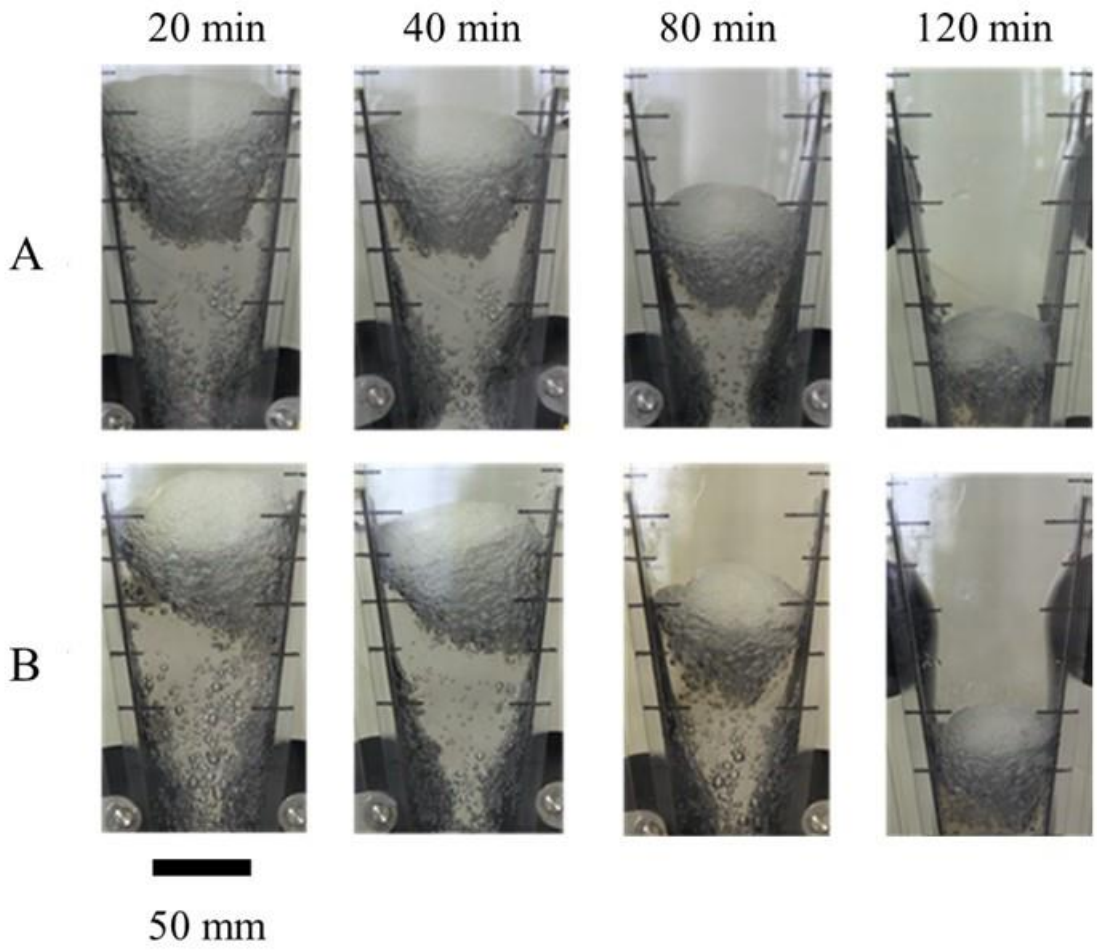

Fig. 3.4 Course of changes in the gel under condition A and condition B

The photographs of bubble-containing gel formed by Sample A-1 shown in Table 3.1 were taken under condition A and condition B shown in Chapter 3.2.4.2.

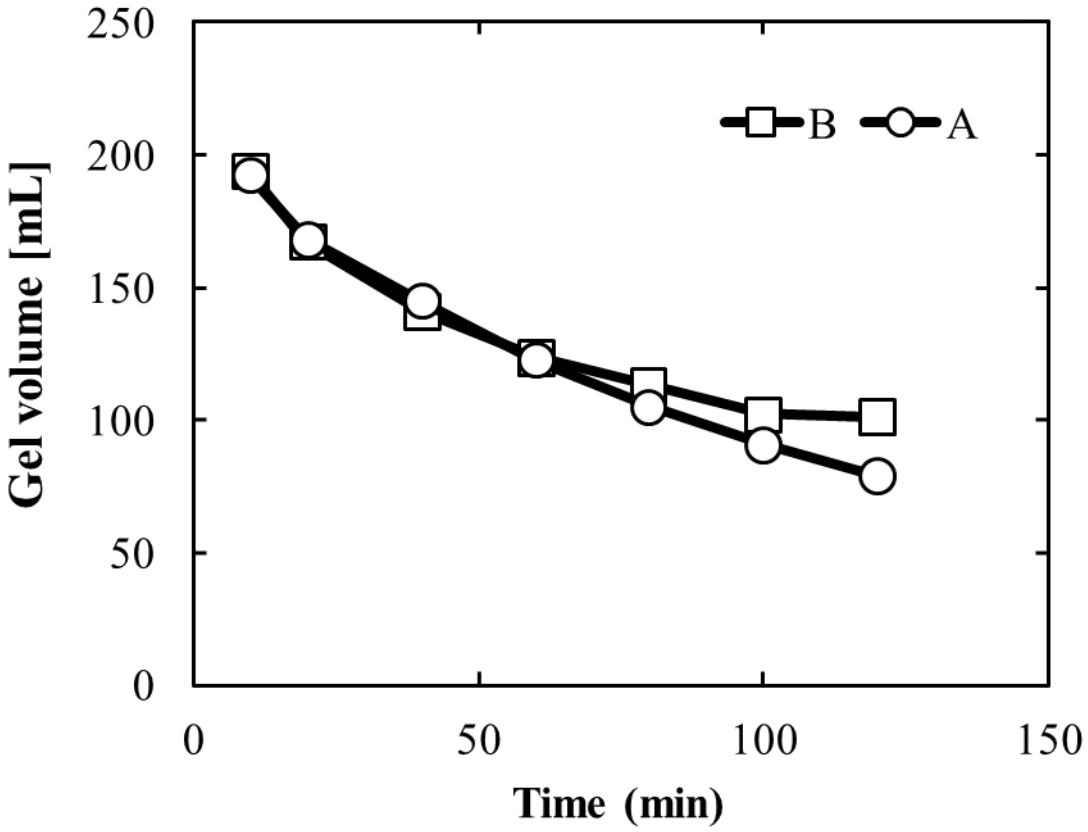

Fig. 3.5 Bubble-containing gel volume (n = 2) under condition A and condition B
The bubble-containing gel volume of Sample A-1 shown in Table 3.1 was measured under condition A and condition B shown in Chapter 3.2.4.2.

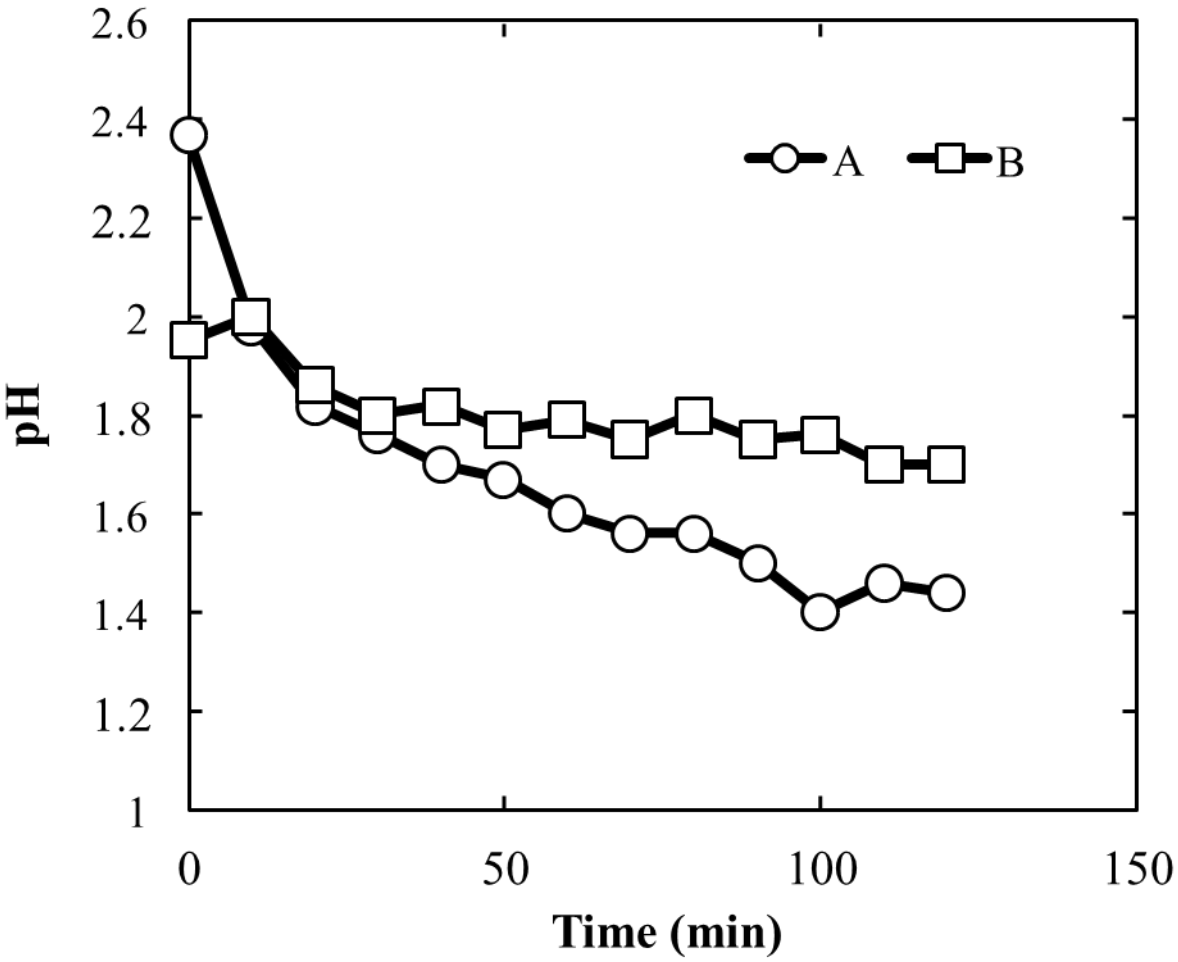

Fig. 3.6 pH in the stomach model under condition A and condition B

The pH in the stomach model after the addition of Sample A-1 shown in Table 3.1 was measured under condition A and condition B shown in Chapter 3.2.4.2.

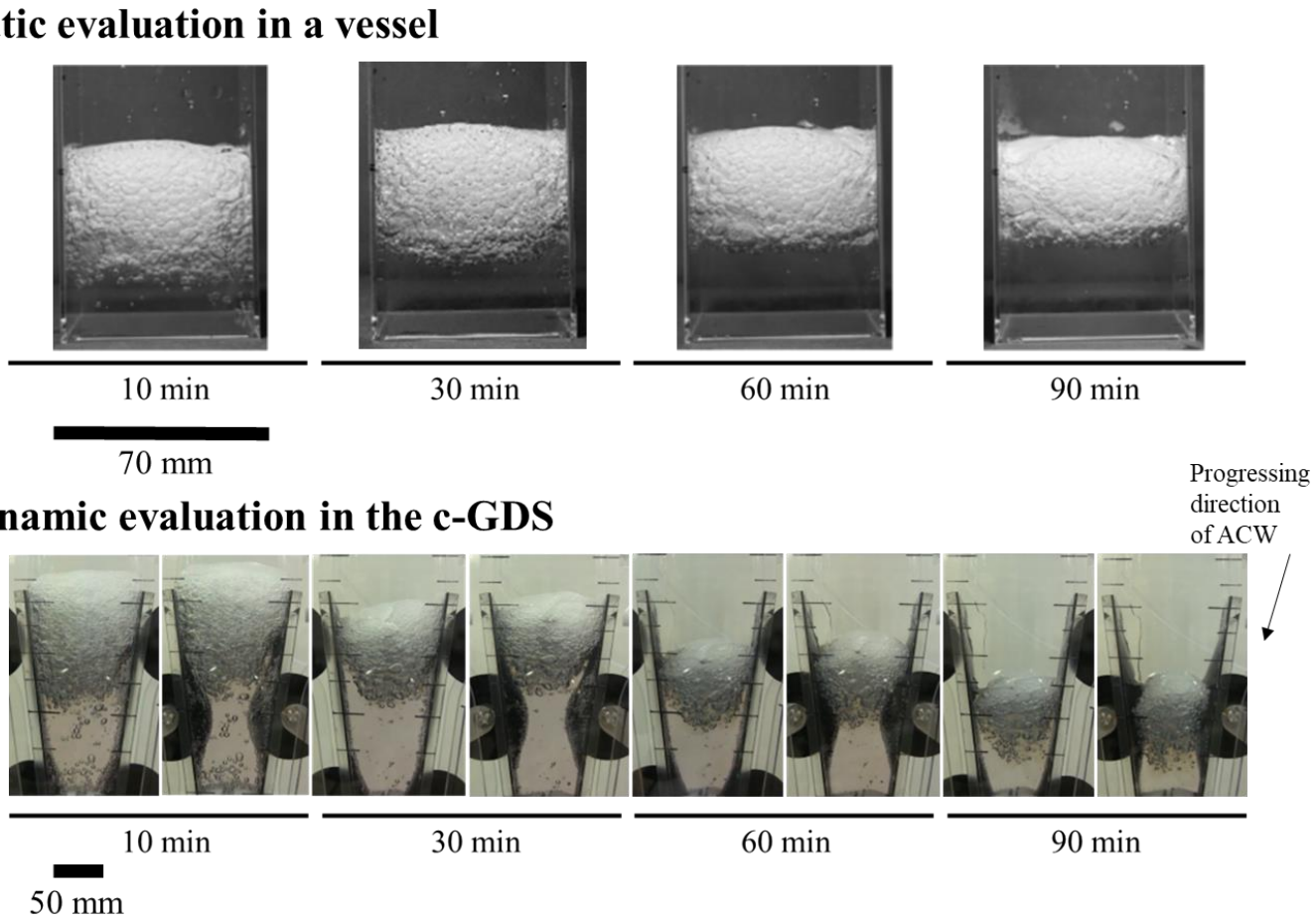

Fig. 3.7 Course of the changes in the gel in the static evaluation in a vessel and the dynamic evaluation using the c-GDS

(a) Photographs of a bubble-containing gel in a vessel a 90-minute period after being mixed with artificial gastric juice

(b) Photographs of bubble-containing gel digested in the c-GDS a 90-minute period after being mixed with artificial gastric juice

Each scale of c-GDS vessel is plotted at 50-mL intervals on the c-GDS vessel.

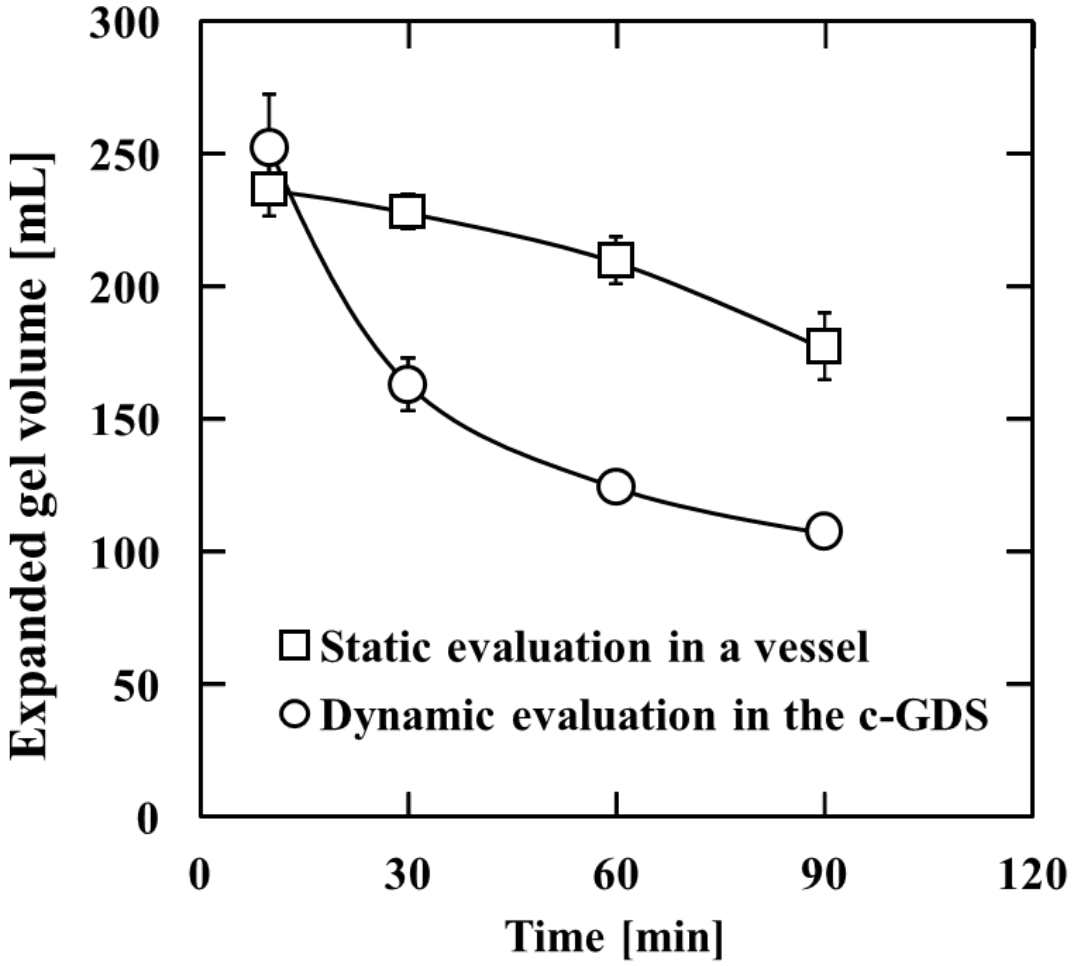

Fig. 3.8 Bubble-containing gel volume (n = 4) in the static evaluation using a vessel and dynamic evaluation using the c-GDS

Gel volume was calculated based on the photographs of the frontal aspect of the vessels, using formula 3.1.

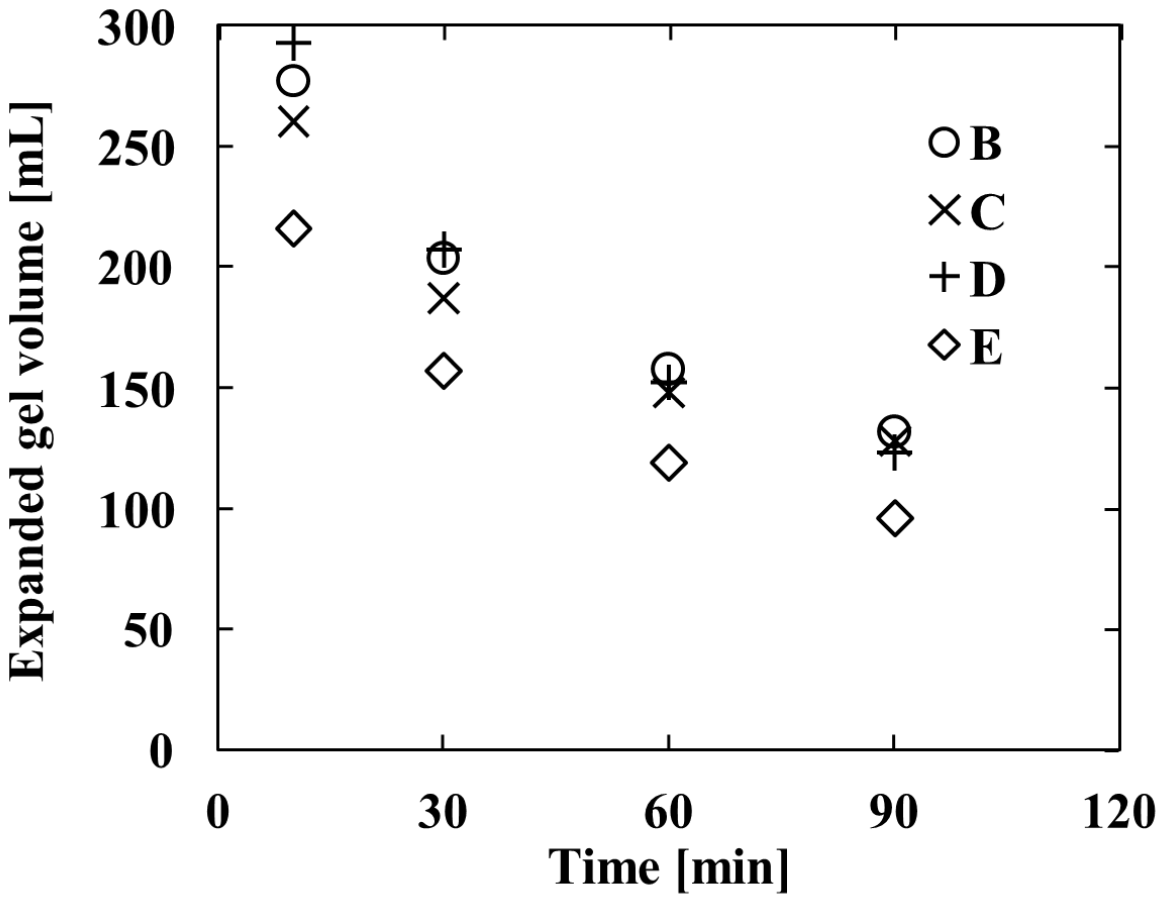

Fig. 3.9 Course of changes in the gel volume according to the pectin type

Gel volume was calculated based on the photographs of the frontal aspect of the vessels, using formula 3.1.

Compositions of Sample B, C, D and E are shown in Table 3.1.

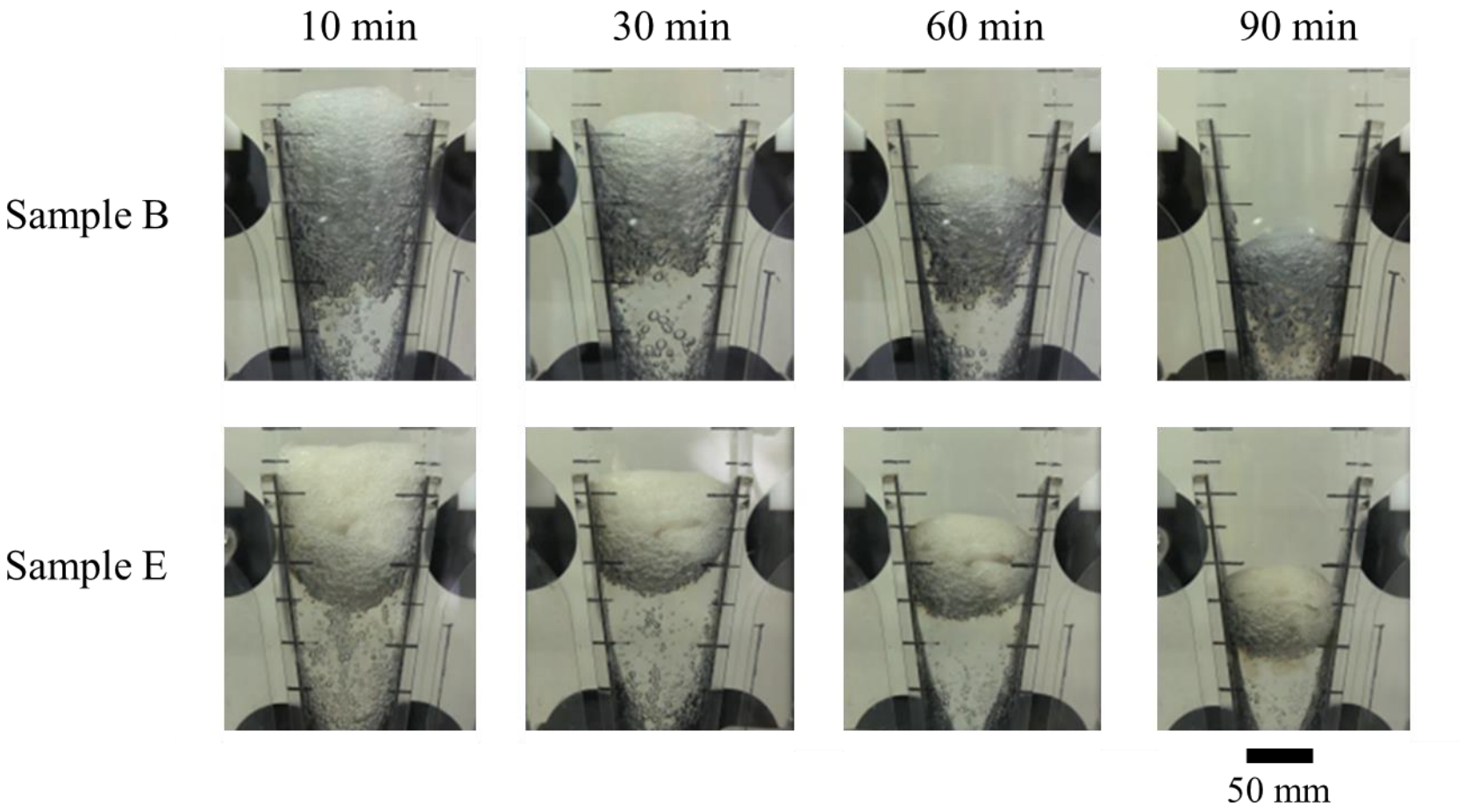

Fig. 3.10 Photographs of the digestive behavior of Sample B and Sample E in the c-GDS

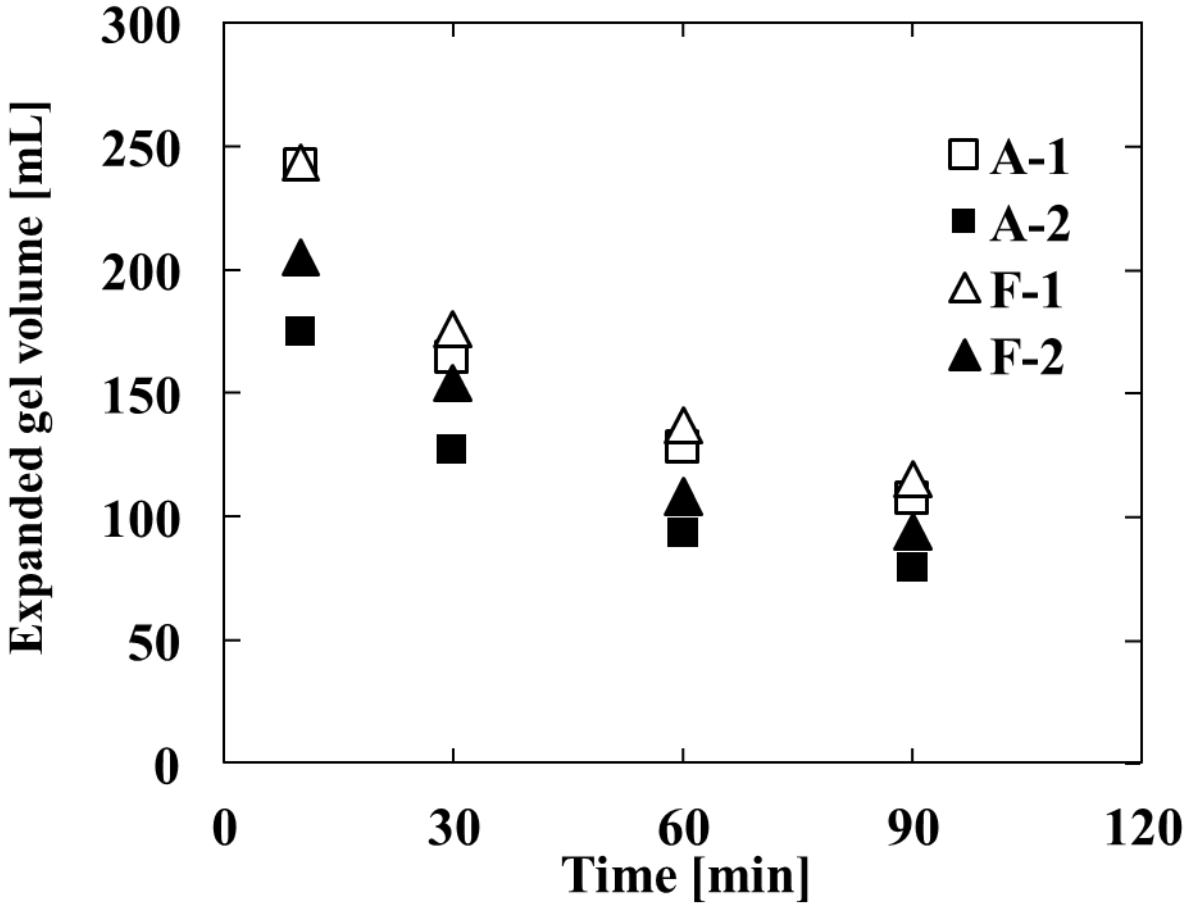

Fig. 3.11 Course of changes in the gel volume according to the citric acid concentration
Gel volume was calculated based on the photographs of the frontal aspect of the vessels, using formula 3.1.
Compositions of Sample A-1, A-2, F-1 and F-2 are shown in Table 3.1.

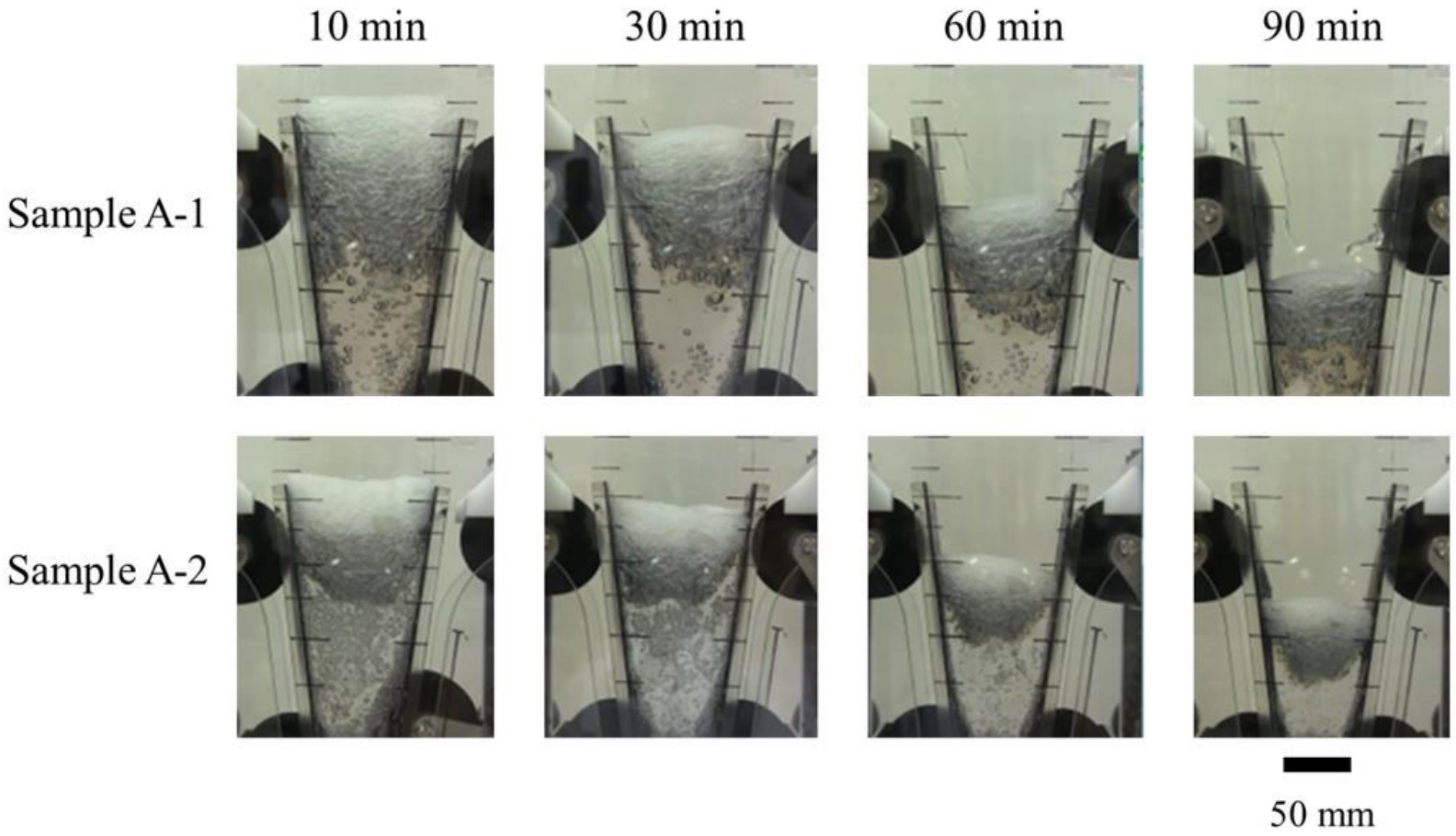

Fig. 3.12 Photographs of the digestive behavior of Sample A-1 and Sample A-2 in the c-GDS

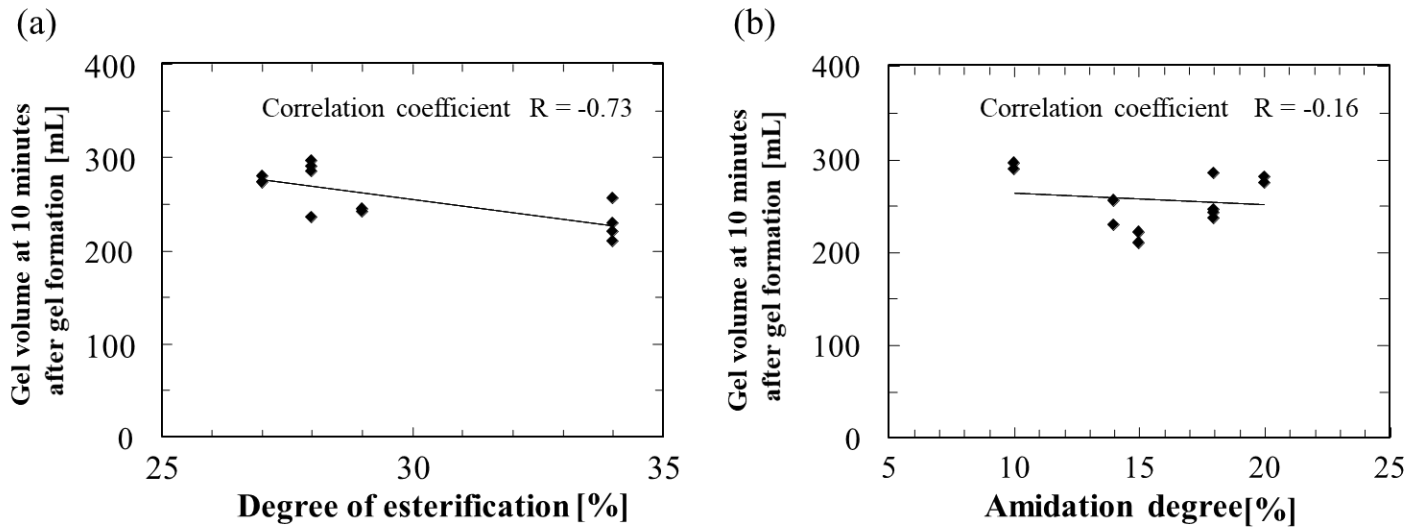

Fig. 3.13 Correlations between the gel volume at 10 minutes after gel formation and each factor

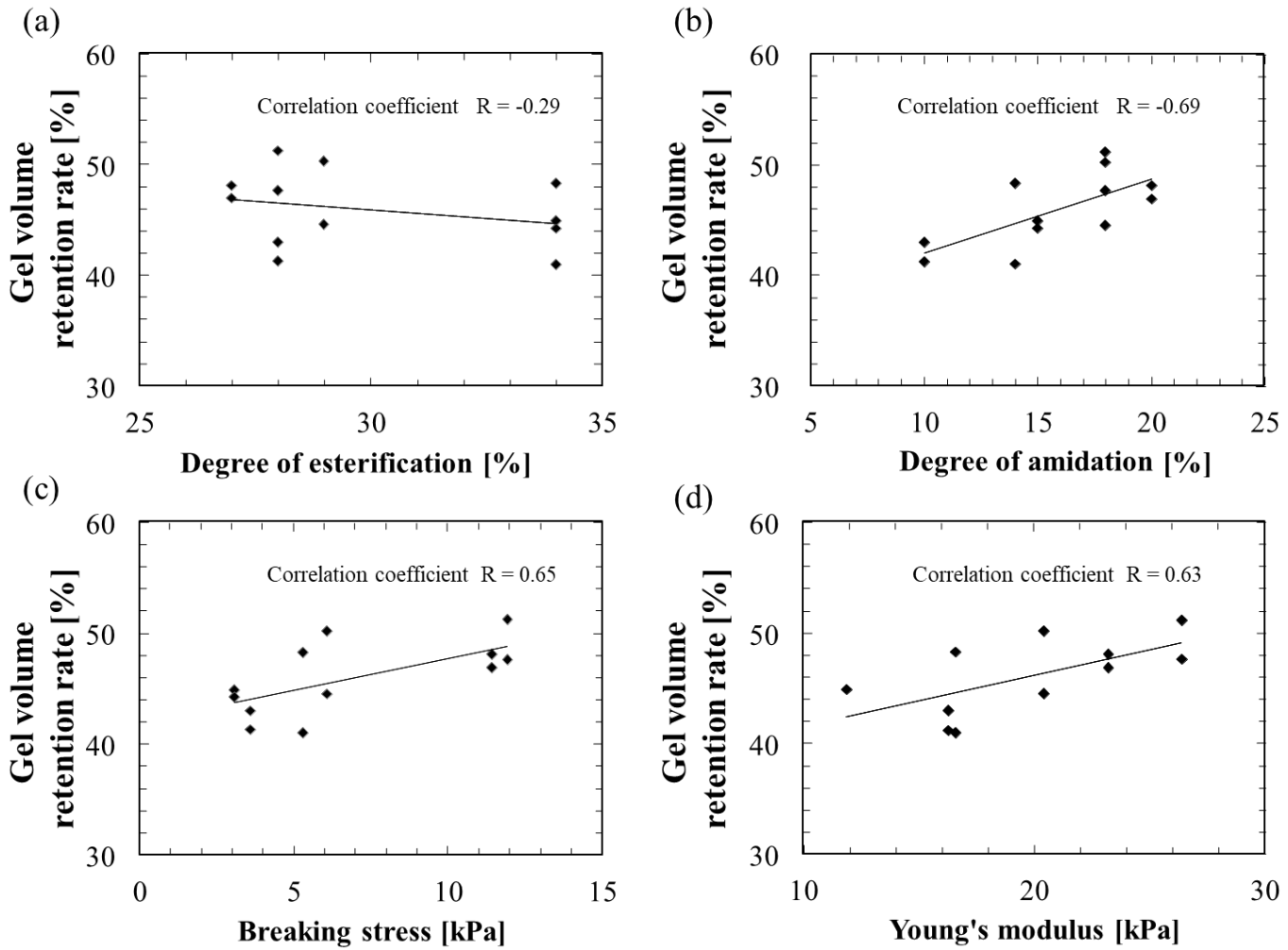

Fig. 3.14 Correlations between the gel volume retention rate and each factor

The formula in 3.2 was used to calculate the gel volume retention rate.

CHAPTER 4. GENERAL CONCLUSIONS

This chapter summarizes and discusses the results described in Chapters 2 and 3 and discusses future research perspectives.

4.1 Conclusions

In this study a beverage that forms a bubble-containing gel in the stomach was developed and evaluated with the intention of applying it to controlling appetite. First, appetite control by stretching the stomach wall was selected as a method that would entail few side effects. Then, issues such as poor ingestibility, persistence, or convenience were identified by reviewing previous studies, and technology used in the pharmaceutical industry was applied to beverages in order to deal with these issues.

The purpose of this thesis was to develop a beverage that forms a bubble-containing gel in the stomach and produces a feeling of satiety, and then to identify factors that affect the gel's expandability and its persistence.

In the part of the study reported in Chapter 2, a beverage that forms a bubble-containing gel when added to artificial gastric juice was developed. The beverage was then tested to determine whether it would form a bubble-containing gel in the stomach and stretch the stomach, and whether it would induce satiety.

First, an optimal ionic polysaccharide was selected to include in this beverage. The results of conducting a test in which a solution containing 6 types of ionic polysaccharides were added to an artificial gastric juice showed that solutions containing 3 of the types of polysaccharides turned into a gel when added. When carbonated solutions containing each of 3 polysaccharides were added to artificial gastric juice, the gel was observed to expand. The results of attempting to identify the most suitable raw material for use in beverages from among the 3 types of polysaccharides showed that LM pectin had the lowest viscosity and high expansion rate and was the most suitable of the 3 for use in beverages. In this way a beverage that forms a bubble-containing gel when added to artificial gastric juice was developed, and clinical trials were performed in part of this study to determine whether the beverage would stretch the stomach wall and induce a feeling of satiety. MRI examinations showed that a bubble-containing gel was generated in the human stomach, that gastric volume was increased by the volume of the bubble-containing gel, and that the stomach wall was stretched more than by water. Moreover, the results of simultaneously performing

a sensory evaluation on the same subject showed that satiety was induced in comparison with the control. In this way, it was possible to develop a beverage that forms a bubble-containing gel in the stomach and produces a feeling of satiety.

The part of the study described in Chapter 3 was conducted with the aim of identifying factors that affect the volume and volume retention capacity of the bubble-containing gel. The c-GDS test conditions used to evaluate the gastric digestion behavior of a bubble-containing gel and evaluate beverage samples containing different pectin raw materials and citric acid concentration were designed to achieve that aim.

First, the evaluation conditions of the beverage developed were assessed by using a recently developed gastro-digestion simulator. Based on the results of clinical tests of beverage containing foam, the gastric juice secretion and emptying rates were set under two conditions, and the tests were conducted on the beverage developed. Conditions closer to those in the human stomach were selected based on the results of past clinical trials. This dynamic evaluation system was compared with the static evaluation, and the results showed that the volume of the bubble-containing gel decreased larger than static evaluation. Since the result of c-GDS test was closer to the clinical test result, it was considered that the c-GDS is more appropriate.

The c-GDS test conditions were designed based on clinical trial reports as described above. This c-GDS evaluation system was used to assess the effects of pectin raw material parameters and citric acid concentrations on the volume of the bubble-containing gels and their retention capacity. The results showed that the degree of pectin esterification and the citric acid concentration affected the gel volume immediately after reaction of the beverage with gastric juice. They also showed that the degree of amidation and the gel properties affected the gel volume retention capacity. In order to maximize the volume of the bubble-containing gel and increase the volume retention capacity, it is necessary to consider the degree of esterification and degree of amidation of pectin and the concentration of citric acid in the formulation.

There was a difference between the results obtained in vitro and in the clinical trial reported in Chapter 2 and Chapter 3. As shown in Fig. 3.8, the *in vitro* test showed that the volume of the bubble-containing gel 10 minutes after the sample was added to the artificial gastric juice was about 250 mL, whereas, as shown in Fig. 2.4, the clinical test showed that the volume was about 150 mL. Various causes are responsible for the difference. The first is the composition and amount of artificial gastric juice. A 50 mL volume of artificial gastric fluid disintegration test solution 1 (pH 1.2), which is often used in evaluations of

pharmaceuticals, was used in the c-GDS test. On the other hand, there are known to be very large variations in human gastric pH and gastric fluid volume (Kararli, 1995). In fact, the average volume of gastric juice before sample ingestion in the MRI test was approximately 40 mL, and the individual differences were wide, ranging from 2.3 mL to 75.3 mL. The composition of the first disintegration test solution (Japan Pharmacopoeia 1st fluid, pH 1.2) is simple, consisting only of sodium chloride and hydrochloric acid, whereas human gastric juice contains other electrolytes and the digestive enzyme pepsin. Such differences in composition and volume of gastric juice may have affected the volume of the bubble-containing gel. Since the top of the stomach vessel in the c-GDS is open to the atmosphere, even if the bubble-containing gel were compressed by peristaltic movements of side walls, the gel would be pushed out upward. On the other hand, the volume of the intragastric gas measured by MRI was very small, and when the contents of the human stomach are compressed by peristaltic movements of gastric wall, the bubbles may collapse more.

In addition, the satiety inducing effect of the beverage developed in this study should be examined carefully. Since, as described in Chapter 2, the sample used for sensory evaluation contained fructose, high-fructose corn syrup, indigestible dextrin, and fragrances, the possibility that they affected the results of the sensory evaluation cannot be ruled out. In order to accurately verify whether the bubble-containing gel was affected, it will be necessary to evaluate beverage samples that differ only according to whether or not they contain pectin. As described in Chapter 3, since pectin is a natural product, it has a complex steric structure and wide molecular weight distribution, and thus factors other than degree of esterification and degree of amidation may affect gel volume and volume retention capacity. In order to accurately evaluate the factors that affect gel volume and volume retention capacity, it will be necessary to use well characterized materials such as synthetic polymers that can be prepared with different properties.

Finally, a carbonated beverage containing pectin that gel at low pH in the stomach was developed in this study. The clinical trials revealed that when the beverage developed was ingested, a bubble-containing gel formed in the stomach, stretched the stomach wall, and induced satiety. In addition, a c-GDS was used to set conditions that simulated the physical and chemical environment of the human stomach, and beverages having various compositions were evaluated. The results identified citric acid concentration, the raw material species of pectin, the degree of esterification, and the degree of amidation as factors that affect the expansibility and persistence of the bubble-containing gel. In

addition, the influence of these factors on the volume and its retention capacity of the bubble-containing gel was investigated. Thus, a part of the design guidelines for an appropriate beverage composition was examined. The findings obtained in this study are expected to contribute to the development of beverages that produce a feeling of satiety, and to contribute to the prevention of obesity and various obesity-related diseases.

4.2 Future Perspectives

A beverage with novel physical properties achieved by combining the gelation ability of ionic polysaccharides and the gel expanding ability of carbon dioxide was developed in this study, and some of the factors that affect the bubble-containing gel volume and persistence were identified. The beverage developed in this study will provide the basis for technology that can be developed in various directions in the future. There are four possible directions that future development might take.

The first possible direction is toward improvement of the stomach wall stretching and satiety inducing effects. The effects of the pectin ingredients and citric acid concentrations, which can be changed almost freely in the formulation design, and many other components that can be compounded in a beverage were assessed in this study. If how such components affect the gel expansion rate during gelation by gastric fluid and the retention capacity of the gel can be determined, it might be possible to further improve the stomach wall stretching and satiety-inducing effects.

In fact, registered patent has been reported that the breaking strength of a gel is improved by specific ingredients such as hydrogenated castor oil, dextrin, amino acid and protein (Domoto *et al*., 2013 a, b & c). These combinations can be expected to induce greater stomach wall stretching and satiety.

There have also been various reports on the effects of food ingredients on the induction of satiety as well as on stretching of the stomach wall. For example, it has been reported that unsaturated fatty acids (Maljaars *et al*., 2009), potato extract (Chen *et al*., 2012), polydextrose (Alvin *et al*., 2016), and barley β-glucan (Clegg *et al*., 2019) induce satiety. Since the mechanisms of action of these components on satiety are different from the mechanism of action of stretching the stomach wall, a synergistic effect might be achieved by combining them.

The second possible direction is toward technological development of various formulation types. Although the beverage form has the advantage of being able to be ingested very easily, there are some aspects of beverages that are inferior to solid food in

terms of portability and economy. However, this problem may be solved if a concentrated beverage type or powder type formulation with design technology that is excellent in terms of portability and economy can be developed. Such a formulation could be used in various ways, such as by ingesting as is, or dissolving in water or carbonated water.

The third possible direction is toward application of pharmaceutical technology to the controlled release of active ingredients. Such technology was first used for drug delivery systems in the pharmaceutical industry, but it has hardly ever been applied to the controlled release of ingredients in food. When a beverage gels in the stomach, release of its ingredients is delayed. The release rate will depend on the gel size, density of gel network and molecular weight of ingredients. Thus, if this technology were applied to food design, it would possible to control the delivery rate of food ingredients into the small intestine by controlling its release from the gel formed in the stomach. For example, it might be possible to increase the sustainability of the satiety-inducing effect by including slow-releasing components that affect blood glucose levels, such as dextrin. Moreover, the bioavailability of hydrophobic bioactive food ingredients such as some vitamins may be increased by delivering them into the small intestine little by little.

The fourth possible direction is toward a full-time contribution on the improvement of consumer lifestyle to obesity prevention. The purpose of the present study was to develop and evaluate beverages. However, it is not enough to provide products to improve the behavioral factors that lead to obesity. In order to change consumer lifestyle it will be necessary to combine provision of services with dietary guidance and provision of lifestyle improvement programs, including exercise and sleep improvement programs. Doing so will require the development of technologies that match effective services.

The technology developed in this thesis still require research. Since this technology have a new concept in the food industry, there are various applications other than those listed above. I hope that various technologies and products that contribute to people's health will be developed based on this research.

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LIST OF PUBLICATIONS

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