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Effects of Food Containing Benzyl Glucosinolate on Bowel Movement and Intestinal Environment in Healthy Japanese:

A Randomized, Placebo-controlled, Double-blind, Crossover Study

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● Abstract

Objectives: The objective of this study is to evaluate how the ingestion of food containing papaya extract which includes benzyl glucosinolate, contributes to the improvement of bowel movements and intestinal environment of individuals with the tendency for constipation and those with the normal level of defecation.
Methods: A randomized, placebo-controlled, double-blind crossover clinical study was conducted to elucidate the effect of the ingestion of the food. In this study we have measured the bowel movements frequency, number of days which the subjects defecated, volume of the stool, and the intestinal flora as the primary outcome. In addition, we have also evaluated fecal properties such as scent, texture, as well as its color as the secondary outcome. A questionnaire was provided to the subjects during the study in order to monitor adverse events.
Results: 50 subjects made a start with the intervention (non-ingestion period). 19 subjects were discontinued from the clinical study and the remaining 31 subjects continued to take part in the study until its completion. 1 was removed from the study due to inadequate bowel movements recorded, thus data obtained from 30 subjects was used for the analysis of efficacy. After 2-week of ingestion, the intergroup analysis showed significant differences in "Frequency of bowel movement" and "Volume of stool" in contrast to those who had ingested the Placebo. The share in some of the intestinal flora had changed significantly between the two groups after 2-week of ingestion. No adverse events were observed after the ingestion of the test product.
Conclusion: It was determined that the ingestion of the food containing papaya extract which includes benzyl glucosinolate for a 2-week period maintained *Faecalibacterium prausnitzii* share which was decreased caused by poor diet, and improved the subjects' intestinal environment and their bowel movements. In addition, no safety-related matter occurred during the course of the study.

Key Words: papaya, benzyl glucosinolate (BGL), benzyl isothiocyanate (BITC), myrosinase, Caricaceae, Brassicales, bowel movement, intestinal flora

1. INTRODUCTION

The importance of intestinal bacteria is well known within the healthcare industry. There are approximately 100 trillion if not more intestinal bacteria inside the human intestine[1)], as a result of the interaction with the host, it builds an environmental system called ecosystem[2)]. Recent analysis of the intestinal flora utilizing new techniques such as Next Generation Sequencing (NGS) has revealed that digestive tract disorder, diabetes, obesity, allergic diseases, neuropsychiatric disorder, arteriosclerosis, and cancer are closely related to the disorder called dysbiosis which is the breakdown in the balance of intestinal bacteria[3)].
Papaya- particularly unripe green papaya, has gained popularity as a superfood recently. It was reported that benzyl isothiocyanate (BITC) and its precursor benzyl glucosinolate (BGL), extracted from papaya or watercress, have anti-microbial activity and suppress colon cancer cells[4)-9)]. Therefore we decided to focus on the relationship between papaya and intestinal bacteria; and how intestinal bacteria is affected when Papaya is ingested, a randomized, placebo-controlled, double-blind, crossover study was conducted to verify the effects and safety of food mainly containing BGL extracted from papaya on bowel movements and the intestinal flora in individuals with the tendency for constipation and those with the normal level of defecation.

1) JACTA (Japan Clinical Trial Association)
2) Nihonbashi M's Clinic
3) Fuji Sangyo Co., Ltd.

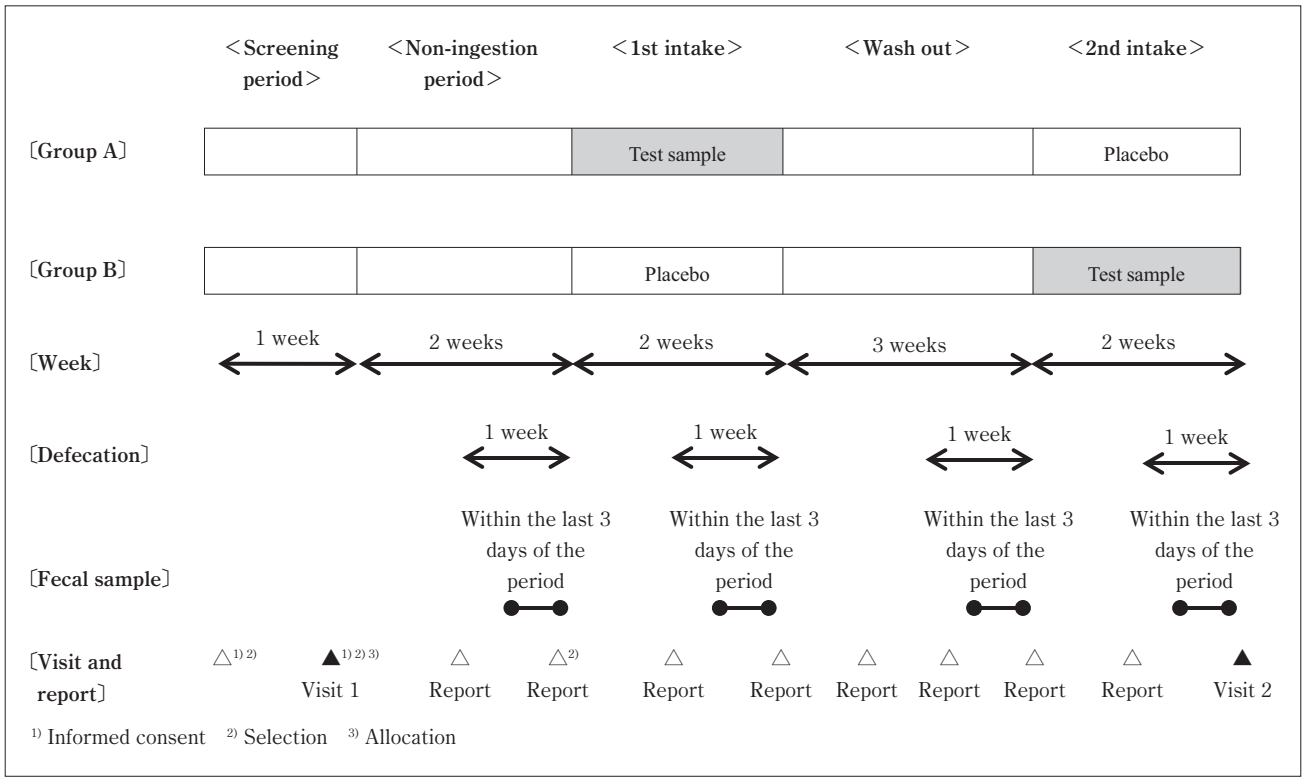

Figure 1 Schedule for the study

2. METHODS

2.1. Trial design

A randomized, placebo-controlled, double-blind crossover study was conducted with the aid of a fund from Fuji Sangyo Co., Ltd. (Kagawa) at Japan Clinical Trial Association (JACTA, Tokyo). The clinical study took place from August 19th, 2017 to January 19th, 2018. During this period, the subjects were divided into three groups with different testing sessions. The implementation period consisted of 10 weeks, a 1-week screening period, followed by 2 weeks non- ingestion period, 2 weeks intervention (1st), 3 weeks of wash out period, and 2 weeks intervention (2nd) (**Figure 1**). This study was conducted in accordance with the ethical principles of the declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all Subjects. This trial was registered at UMIN Clinical Trial Registry (Trial ID: UMIN000028696). Subjects were assigned to the test groups accordingly by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subject

Healthy subjects participated in the present study. All of the subjects in this study were volunteers who had enrolled in the monitor bank of TRIBELATE CORPORATION (Tokyo), recruited from July through October 2017.

2.2.1. Inclusion criteria

(1) Healthy Japanese males and females from 20 to 59 years of age;
(2) Those who bowel movement is less than or equal to 10 times in two weeks.
The doctor conducting the present study confirmed subjects were not of ill health.

2.2.2. Exclusion criteria

(1) Subjects with chronic constipation;
(2) Subjects with food allergies;
(3) Subjects who are pregnant or have possibility to become pregnant during the study or breast-feeding;
(4) Subjects taking medicinal product which may influence the test results;
(5) Continuous usage of supplements and/or functional foods affecting the test results, including food for specified health uses (FOSHU);
(6) Subjects who are judged as unsuitable for the study by the principle investigator.

2.3. Randomization

From all 118 applicants, 42 were eliminated (27; inclusion/ exclusion criteria, 15; declined with personal

Table 1　Nutritional contents of the test samples (per 1.2 g / daily intake)

Item	Active	Placebo
Energy	4.21 kcal	4.62 kcal
Moisture	0.06 g	0.05 g
Protein	0.11 g	0.00 g
Fat	0.019 g	0.014 g
Ash	0.034 g	0.017 g
Total carbohydrate	0.97 g	1.12 g
Salt equivalent	0.72 mg	0.84 mg

reasons), and 76 were sequentially allocated to Group A and Group B by 38 in each using a random number table. 5 declined after that. In the process of subject assignment, background factors such as gender, age, and frequency of bowel movement were taken into consideration to avoid biased distribution. Subjects in Group A received the active sample first and subjects in Group B received the placebo first for 2 weeks in a double-blind, crossover fashion.

2.4. Description of test foods and blinding

The test product contained papaya extract including benzyl glucosinolate, the active ingredient (so hereinafter the sample was called "Active"). The placebo ("Placebo") does not include BGL. The amount of daily intake was 4 tablets (weighed 1.2 g), which included 0.15 mg of BGL per day. Both tablets were prepared by Fuji Sangyo Co., Ltd., and were indistinguishable in shape, color, smell, or taste, and were managed by an identification symbol. All involved were blinded. **Table 1** shows the nutritional contents of the test samples.

2.5. Experimental procedures

2.5.1. Experimental protocol

Subjects consumed 4 tablets of the supplement with hot or cold water after dinner every day for 2 weeks. Subjects were instructed as follows: to consume the assigned food; to maintain their usual lifestyles and habits; to avoid taking other supplements; to avoid excessive amounts of food, drink, or alcohol; to avoid antiflatulent, cathartic, or food for specified health uses (FOSHU) having an intestinal function; to avoid excessive intake of lactobacillus food (such as yogurt, lactobacillus beverage, and Natto), oligosaccharide, or dietary fiber which may influence fecal flora; to maintain a daily record of one's bowel movement (frequency and volume of stool) and fecal properties (scent, texture, and color); and to provide the diary every week to the study coordinator.

2.5.2. Outcome

The objective of this study is to elucidate the improvement of bowel movements and the influence on intestinal flora by the ingestion of food containing papaya extract including BGL. To evaluate this objective, frequency of bowel movement, days of defecation, volume of stool, and intestinal flora were measured as the primary outcome. As the secondary outcome, fecal properties such as scent, texture, and color were recorded. Moreover, adverse events were collected by means of a written questionnaire during the study to evaluate the safety of the test foods. According to the schedule shown in **Figure 1**, parameters on efficacy and safety of the product were measured. The last piece of data collected during each intake period as a baseline was compared with the data collected after 2-week of ingestion.

2.5.2.1. Bowel movement

Dairy reporting figures of "Frequency of bowel movement", "Days of defecation", and "Volume of stool" were calculated. "Frequency of bowel movement" is the amount of times each subject defecated per week. "Days of defecation" represents the number of days where subjects defecated per week. Regarding "Volume of stool", subjects compared volume to a 5 x 2.5 cm cylindrical object and recorded every defecation, then totaled the amount of 1 week.

2.5.2.2. Intestinal flora

Subjects collected their feces employing a sampling kit containing a preservative solution 4 times; first one, prior test food- intake (at the end of Non- ingestion period), second one just after 2 weeks of 1st intake period, third one after 3 weeks of wash out period, and the fourth one just after 2 weeks of 2nd intake period. Feces excreted were sampled within 72 hours before and after the test period, based on the reason that it has been demonstrated that it takes 36-72 hours for ingested food to be excreted as feces[10], and subjects with constipation defecated once every few or three days[11] (**Figure 1**).

The microorganism analysis of fecal matter was carried out by NIPPON STEEL & SUMIKIN Eco-Tech Corporation. The DNAs were extracted from the sample by using Extrap Soil DNA Kit Plus ver.2 (NIPPON STEEL & SUMIKIN Eco-Tech Corporation), and the gene dosage of eubacteria were quantified by PicoGreen dsDNA Assay Kit (Invitrogen, Thermo Fisher Scientific K.K.), so called Real-time PCR method[12]. The DNA sequence was analyzed by MiSeq (Illumina, Inc)[13] as a next generation sequencer. By means of QIIME pipeline[14], homology search and community analysis were

Table 2 Questionnaire about fecal properties

Item	Choices, and score showed in parentheses	Unit	Valuation method
Scent of stool	1; Very strong (1), 2; Strong (2), 3; A little strong (3), 4; Odorless (4)	Score/1 defecation	Average difference score at 1 defecation for a week
Texture of stool	1; Watery, no solid pieces (3), 2; Fluffy pieces with ragged edges, a mushy stool (2), 3; Soft blobs with clear cut edges (1), 4: Like a sausage or snake, smooth and soft (0), 5; Like a sausage or snake, but with cracks on its surface (1), 6; Sausage-shaped, but lumpy (2), 7; Separate hard lumps, like nuts (3)	Score/1 defecation	● The difference between the best score (0) and measured score ● Average difference score at 1 defecation for a week
Color of stool	1; Yellow (2), 2; Ocher (1), 3; Light brown (0), 4; brown (1), 5; Dark brown (2), 6; Blackish brown (3)	Score/1 defecation	● The difference between the best score (0) and measured score ● Average difference score at 1 defecation for a week

performed with two kind of database, Greengenes[15] and Silva Living Tree[16].

2.5.2.3. Fecal properties

Subjects rated 3 items; "Scent of stool", "Texture of stool", and "Color of stool" every defecation was monitored on an ordinal scale, which is illustrated in **Table 2**. The subjects' assessment of "Texture of stool" was analyzed using the "Bristol stool form scale"[17] modified to Japanese standards. "Color of stool" was done using a color chart. Then the difference between the best score per defecation was calculated and averaged during a week. As for "Scent of stool", high score means better, and for "Texture of stool" and "Color of stool", low score means better.

2.6. Data analysis

Per-protocol set (PPS) was adopted in the study and the sample size was calculated as 24 participants with the following settings: statistical power of 0.8, effect size of 0.6, and significance level of $p < 0.05$. All statistics were expressed as mean ± standard deviation (SD). For frequency of bowel movement, days of defecation, and intestinal flora, paired t-test was used for intergroup comparisons and intragroup analysis. The Wilcoxon signed-rank test was performed for volume of stool and fecal properties. A chi-square test and Student's t-test were used to compare subject backgrounds between groups, repeated measures ANOVA were used to examine both order effects and period effects in crossover method. Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis. The subjects who came under the following criteria of exclusion were eliminated before the allocation list was opened, 1; consumed less than 80% of the expected dose, 2; without adequate record, 3; fell under the exclusion criteria after enrollment or had justifiable reason for exclusion. Statistical analyses were performed using Statcel 4 (Yanai, 2015) and Excel Tokei 2.15 (SSRI). The results were considered significant at a $< 5\%$ level in the two-sided test.

3. RESULTS

3.1. Participant demographics

76 subjects were randomly assigned to an intervention group. 5 subjects declined to participate, and 71 made a start with screening period. During to the screening period 21 were removed due to declination to participate with personal reasons (9), and diagnosis of the doctor (12), thus 50 (Group A; 26, Group B; 24) made a start with intervention (non- ingestion period). The 16 subjects dropped out according to exclusion criteria (6) and declined to participate due to personal reasons (10) in the middle of the non-ingestion period, and 34 launched the ingestion. 3 were withdrawn due to personal reasons, and the remaining 31 subjects completed the study. For the reason of inadequacy of bowel movements recorded, 1 (Group B) was eliminated, thus data obtained with 30 subjects (Group A; 14 [M; 3, F; 11], Group B; 16 [M; 3, F; 13]) was used for the analysis of efficacy (**Figure 2**). The subject's age range was between 30-59 years of age (mean age 46.9 ± 7.8 y.o.). Data sets were analyzed for evaluations on Group A and B, where there was no significant difference in gender, age, frequency of bowel movement, days of defecation, and volume of stool between groups (**Table 3**). The test sample intake rates were 100% (active) and 100% (placebo), respectively.

3.2. Validity of the crossover method

The effects of either intake period for "Frequency of bowel movement", "Days of defecation", and "Volume of stool" were not significant ($p = 0.524$, $p = 0.419$, and $p = 0.925$, respectively). Thus, we conclude that order effects and period effects can be ignored, and the result obtained from the crossover design in the present study can be evaluated appropriately.

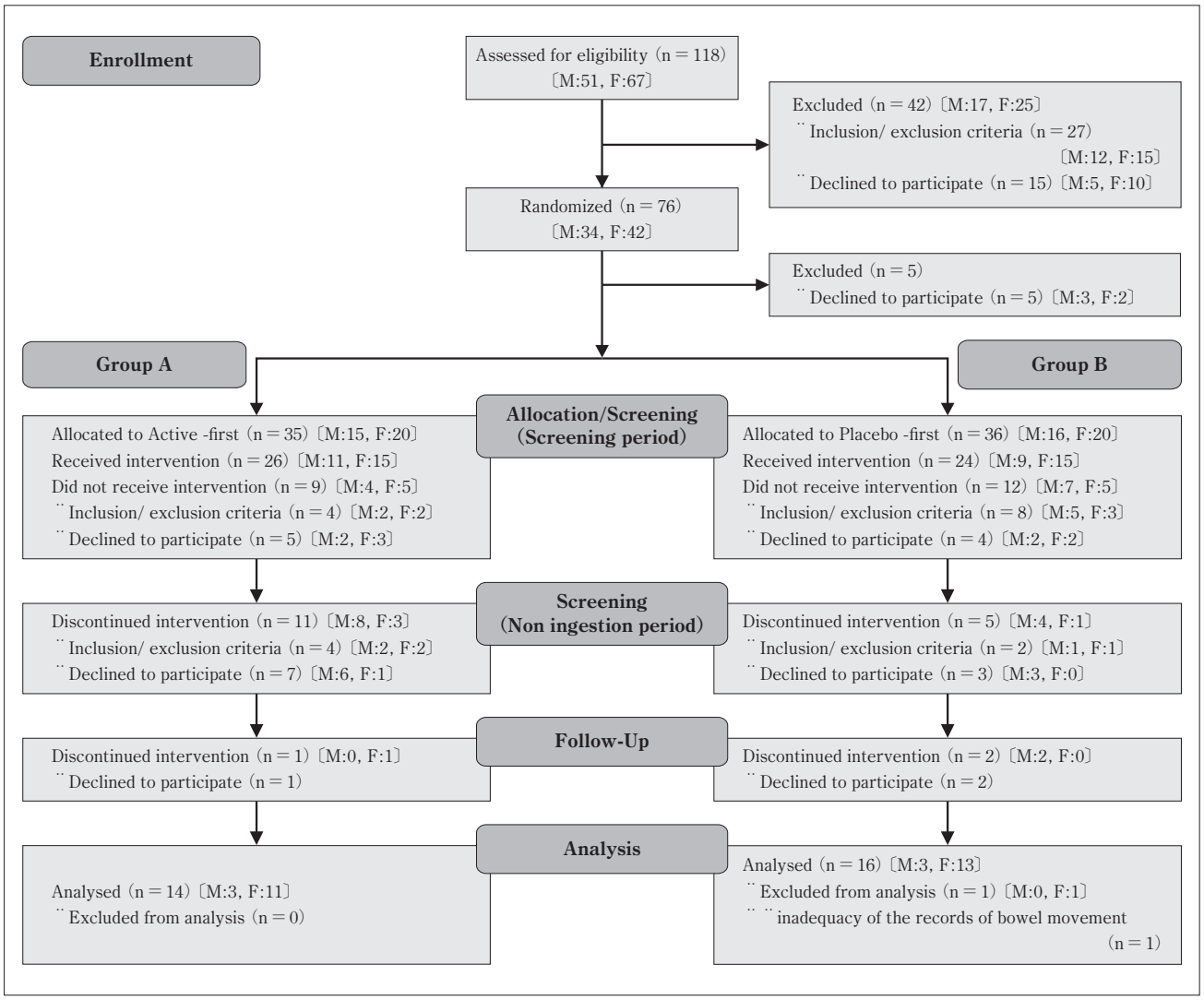

Figure 2　Flow diagram of subject disposition

Table 3　Subject demographics

Item	Unit	Group A	Group B
Subjects	numbers	14	16
Male: Female	numbers	3:11	3:13
Age	years	49.6 ± 5.0	44.6 ± 9.2
Frequency of bowel movement	times/ 1 wk	4.7 ± 2.4	4.1 ± 1.2
Days of defecation	days/1 wk	3.7 ± 1.1	3.8 ± 1.1
Volume of stool	piece/ 1 wk	12.4 ± 8.2	11.0 ± 5.2

Values are expressed as the mean ± SD.
A chi-square test and Student's t-test（compare subject backgrounds between groups）

3.3. Bowel movement

Table 4 depicts the results of bowel movements per week. As for "Frequency of bowel movement" and "Volume of stool", significant differences were listed in the intergroup analysis of either measured value or changes. Regarding "Days of defecation", significant tendency of change was observed between two groups. After 2-week of intake, all items of "Frequency of bowel movement", "Days of defecation", and "Volume of stool" of Active had increased significantly in intragroup analysis, whereas the results for those who received the Placebo showed no significant difference.

Table 4 Results of bowel movement

Item	Unit	Time point	Active (n = 30)	Placebo (n = 30)
Frequency of bowel movement	number/ 1 wk	0-week	4.2 ± 1.9	4.3 ± 1.7
		2-week	5.3 ± 2.4 **	4.7 ± 1.9 #
		⊿ 0-2 w	1.1 ± 1.7	0.4 ± 1.5 #
Days of defecation	day/ 1 wk	0-week	3.6 ± 1.1	3.8 ± 1.1
		2-week	4.3 ± 1.4 **	4.1 ± 1.4
		⊿ 0-2 w	0.7 ± 1.0	0.2 ± 1.2 ‡
Volume of stool	piece/ 1 wk	0-week	11.3 ± 6.6	12.8 ± 6.2
		2-week	15.7 ± 8.1 **	13.9 ± 7.8 #
		⊿ 0-2 w	4.5 ± 5.0	1.1 ± 6.4 ##

Values are expressed as the mean ± SD.
** $p < 0.01$ indicates a significant difference in comparison to 0-week.（"Frequency of bowel movement" and "Days of defecation"；paired t-test, "Volume of stool"；Wilcoxon signed-rank test）
‡ $p < 0.1$, # $p < 0.05$, ## $p < 0.01$ indicates a significant difference between two groups.（"Frequency of bowel movement" and "Days of defecation"；paired t-test, "Volume of stool"；Wilcoxon signed-rank test）

3.4. Intestinal flora

Table 5 shows changes in the composition of the intestinal flora during the study. As for intergroup analysis, the "*Faecalibacterium prausnitzii*"(*Clostridium* cluster IV) share, the "*Clostridium nexile*"(*Clostridium* subcluster XIVa), and the "*Ruminococcus obeum*"(*Clostridium* subcluster XIVa) share showed significant differences. Other parameters did not show any significant difference between groups. "*Faecalibacterium prausnitzii*"(*Clostridium* cluster IV) share of Placebo significantly decreased within the group, while "*Clostridium nexile*"(*Clostridium* subcluster XIVa) or the "*Ruminococcus obeum*"(*Clostridium* subcluster XIVa) showed no significant differences in intragroup analysis after 2 weeks.

3.5. Fecal properties

Table 6 depicts the results of the state of stool. All items showed no significant difference in the intergroup analysis, whereas "Color of stool" of Active indicated upward significantly, and "Texture of stool" of Active tended to be proper after 2-week of ingestion.

3.6. Safety

No adverse events associated with the ingestion of test product were observed in the course of the reporting.

4. DISCUSSION

A randomized, placebo-controlled, double-blind, crossover study was conducted to verify the effect of the ingestion of food mainly made from papaya extract which includes BGL on bowel movement and intestinal flora of individuals with the tendency for constipation and those with the normal level of defecation. As for the primary outcome, the intergroup analysis showed significant differences of bowel movement on "Frequency of bowel movement" and "Volume of stool" and in the share in the intestinal flora of "*Faecalibacterium prausnitzii*", "*Clostridium nexile*", and "*Ruminococcus obeum*", after 2-week of ingestion. On the secondary outcome, fecal properties such as scent, texture, and color did not show significant difference between two groups. Moreover, it proved that no abnormal change was triggered by the ingestion of the test product.

Main Findings

It was found out that intake of BGL improved the bowel movement and contributed to maintaining good intestinal environment. Papaya is the fruit of a plant that belongs to the order Brassicales and family Caricaceae, and its scientific name is *Carica papaya* L. On the other hand, glucosinolate is a kind of sulfur containing compound which is contained in cruciferous vegetables such as cabbage or broccoli and reveals to affect intestinal flora[18]. In particular the unripe papaya or papaya seeds are rich in BGL which has anticancer properties[19] and lipid antioxidation[20]. Moreover, it is well known that the BTIC produced from BGL by myrosinase activity encourages the function of detoxification enzymes[21] and exerts anticancer effect[22)23)]. Many studies suggest that the BTIC contained in papaya provides anti-inflammatory[24] and anti-allergic effect[25]. According to the latest report, Yanaka (2018) found that sulforaphane glucosinolate (SGS) contained in broccoli sprouts improved the bowel habits of healthy human subjects[26]. It was indicated that SGS enhanced antioxidant enzyme activity and protected the gastrointestinal tract from chronic oxidative stress in daily life. In the light of the above facts, it was inferred that bowel movements were improved owing to the antioxidant effect of BGL contained in papaya. In addition, it was showed that oxidative stress caused

Table 5　Results of intestinal flora

No.	Item	Time point	Active（n = 30）	Placebo（n = 30）
1	*Abiotrophia*（Lactobacillales）	0-week	0.001 ± 0.002	0.001 ± 0.002
		2-week	0.000 ± 0.001	0.000 ± 0.001
		⊿ 0-2 w	0.000 ± 0.002	0.000 ± 0.002
2	*Aerococcus*（Lactobacillales）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
3	*Facklamia*（Lactobacillales）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
4	*Carnobacterium*（Lactobacillales）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.001	0.000 ± 0.001
		⊿ 0-2 w	0.000 ± 0.001	0.000 ± 0.001
5	*Granulicatella*（Lactobacillales）	0-week	0.008 ± 0.008	0.011 ± 0.015
		2-week	0.009 ± 0.010	0.012 ± 0.014
		⊿ 0-2 w	0.001 ± 0.007	0.001 ± 0.010
6	*Trichococcus*（Lactobacillales）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
7	*Enterococcus*（Lactobacillales）	0-week	0.045 ± 0.128	0.020 ± 0.042
		2-week	0.038 ± 0.100	0.110 ± 0.305
		⊿ 0-2 w	− 0.007 ± 0.125	0.089 ± 0.306
8	*Lactobacillus*（Lactobacillales）	0-week	0.763 ± 0.958	0.863 ± 1.046
		2-week	0.633 ± 0.746	0.811 ± 1.484
		⊿ 0-2 w	− 0.130 ± 0.723	− 0.052 ± 1.151
9	*Pediococcus*（Lactobacillales）	0-week	0.000 ± 0.000	0.005 ± 0.029
		2-week	0.000 ± 0.001	0.002 ± 0.008
		⊿ 0-2 w	0.000 ± 0.001	− 0.003 ± 0.021
10	*Leuconostoc*（Lactobacillales）	0-week	0.011 ± 0.051	0.001 ± 0.003
		2-week	0.002 ± 0.006	0.001 ± 0.002
		⊿ 0-2 w	− 0.010 ± 0.051	0.000 ± 0.003
11	*Weissella*（Lactobacillales）	0-week	0.001 ± 0.004	0.003 ± 0.014
		2-week	0.000 ± 0.000	0.001 ± 0.004
		⊿ 0-2 w	− 0.001 ± 0.004	− 0.002 ± 0.015
12	*Lactococcus*（Lactobacillales）	0-week	0.005 ± 0.018	0.003 ± 0.008
		2-week	0.007 ± 0.018	0.003 ± 0.009
		⊿ 0-2 w	0.002 ± 0.024	0.000 ± 0.012
13	*Streptococcus*（Lactobacillales）	0-week	1.521 ± 4.207	0.950 ± 1.295
		2-week	1.434 ± 3.603	0.754 ± 1.043
		⊿ 0-2 w	− 0.087 ± 1.570	− 0.196 ± 1.242
14	*Faecalicoccus*（Lactobacillales）	0-week	0.023 ± 0.035	0.029 ± 0.073
		2-week	0.024 ± 0.039	0.031 ± 0.054
		⊿ 0-2 w	0.001 ± 0.027	0.002 ± 0.044
15	Total of No.1-14（Total of Lactobacillales）	0-week	2.378 ± 4.197	1.887 ± 1.740
		2-week	2.147 ± 3.733	1.726 ± 2.240
		⊿ 0-2 w	− 0.231 ± 1.678	− 0.161 ± 1.916
16	*Bifidobacterium*	0-week	10.100 ± 9.431	8.240 ± 7.480
		2-week	10.691 ± 10.993	11.110 ± 9.329 *
		⊿ 0-2 w	0.592 ± 8.649	2.870 ± 7.502
17	*Bacteroides*	0-week	9.186 ± 7.607	8.117 ± 4.785
		2-week	9.168 ± 6.930	8.339 ± 6.550
		⊿ 0-2 w	− 0.018 ± 5.026	0.222 ± 5.404
18	*Prevottella*	0-week	0.864 ± 1.536	1.268 ± 2.565
		2-week	1.469 ± 3.540	1.187 ± 2.569
		⊿ 0-2 w	0.605 ± 3.111	− 0.082 ± 2.075
19	*Streptococcus*	0-week	1.521 ± 4.207	0.950 ± 1.295
		2-week	1.434 ± 3.603	0.754 ± 1.043
		⊿ 0-2 w	− 0.087 ± 1.570	− 0.196 ± 1.242
20	*Lactobacillus*	0-week	0.763 ± 0.958	0.863 ± 1.046
		2-week	0.633 ± 0.746	0.811 ± 1.484
		⊿ 0-2 w	− 0.130 ± 0.723	− 0.052 ± 1.151

Table 5 Results of intestinal flora

No.	Item	Time point	Active（n = 30）	Placebo（n = 30）
21	*Enterococcus*	0-week	0.045 ± 0.128	0.020 ± 0.042
		2-week	0.038 ± 0.100	0.110 ± 0.305
		⊿ 0-2 w	− 0.007 ± 0.125	0.089 ± 0.306
22	*Clostridium*	0-week	3.932 ± 2.487	3.754 ± 1.901
		2-week	4.044 ± 2.636	4.490 ± 3.479
		⊿ 0-2 w	0.112 ± 2.217	0.736 ± 2.526
23	*Ruminococcus*	0-week	10.899 ± 9.320	9.293 ± 8.917
		2-week	10.460 ± 9.565	9.808 ± 6.975
		⊿ 0-2 w	− 0.439 ± 4.304	0.515 ± 5.995
24	*Eubacterium*	0-week	14.107 ± 7.061	17.390 ± 8.050 #
		2-week	14.391 ± 7.154	14.928 ± 8.726 †
		⊿ 0-2 w	0.285 ± 6.492	− 2.462 ± 6.611
25	*Roseburia*	0-week	0.679 ± 0.897	0.680 ± 0.831
		2-week	0.474 ± 0.609 *	0.651 ± 0.693
		⊿ 0-2 w	− 0.205 ± 0.538	− 0.029 ± 0.944
26	*Catenibacterium*	0-week	0.646 ± 2.347	0.614 ± 1.973
		2-week	0.671 ± 2.363	0.796 ± 2.683
		⊿ 0-2 w	0.025 ± 0.127	0.182 ± 0.987
27	*Fusobacterium*	0-week	0.148 ± 0.492	0.073 ± 0.168
		2-week	0.247 ± 0.611	0.649 ± 2.692
		⊿ 0-2 w	0.099 ± 0.463	0.576 ± 2.641
28	*Dialister*	0-week	0.629 ± 1.153	0.688 ± 1.307
		2-week	0.724 ± 1.182	0.611 ± 1.101
		⊿ 0-2 w	0.095 ± 0.550	− 0.078 ± 0.616
29	*Veillonella*	0-week	0.225 ± 0.874	0.266 ± 0.759
		2-week	0.266 ± 0.954	0.276 ± 0.880
		⊿ 0-2 w	0.040 ± 0.172	0.010 ± 0.668
30	*Megaspharea*	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
31	*Escherichia*	0-week	0.138 ± 0.344	0.082 ± 0.192
		2-week	0.120 ± 0.275	0.075 ± 0.137
		⊿ 0-2 w	− 0.018 ± 0.344	− 0.007 ± 0.207
32	Total of No.16-31	0-week	53.882 ± 12.318	52.299 ± 10.264
		2-week	54.832 ± 11.875	54.594 ± 11.343
		⊿ 0-2 w	0.950 ± 9.029	2.295 ± 8.689
33	*C. orbiscindens*（*Clostridium* cluster IV）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
34	*Faecalibacterium prausnitzii*（*Clostridium* cluster IV）	0-week	8.215 ± 7.148	9.596 ± 6.105
		2-week	8.082 ± 6.112	6.742 ± 4.812 **
		⊿ 0-2 w	− 0.134 ± 5.231	− 2.854 ± 4.845 #
35	*Ruminococcus bromii*（*Clostridium* cluster IV）	0-week	1.979 ± 3.408	2.135 ± 3.724
		2-week	2.476 ± 3.276	3.015 ± 5.011 †
		⊿ 0-2 w	0.497 ± 3.433	0.880 ± 2.549
36	Total of No.33-35（Total of *Clostridium* cluster IV）	0-week	10.194 ± 7.729	11.731 ± 6.924
		2-week	10.558 ± 6.991	9.757 ± 7.781 †
		⊿ 0-2 w	0.364 ± 5.311	− 1.974 ± 5.840 ‡
37	*C. clostridioforme*（*Clostridium* subcluster XIVa）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
38	*C. indolis*（*Clostridium* subcluster XIVa）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
39	*C. nexile*（*Clostridium* subcluster XIVa）	0-week	0.064 ± 0.145	0.029 ± 0.077
		2-week	0.047 ± 0.149	0.050 ± 0.144
		⊿ 0-2 w	− 0.017 ± 0.087	0.020 ± 0.077 #
40	*Eubacterium eligens*（*Clostridium* subcluster XIVa）	0-week	0.548 ± 1.024	0.627 ± 1.265
		2-week	0.647 ± 1.464	0.490 ± 1.077 †
		⊿ 0-2 w	0.099 ± 0.662	− 0.138 ± 0.723

Table 5　Results of intestinal flora

No.	Item	Time point	Active（n = 30）	Placebo（n = 30）
41	*Eubacterium hallii*（*Clostridium* subcluster XIVa）	0-week	1.322 ± 0.986	1.387 ± 1.375
		2-week	1.587 ± 2.368	1.144 ± 0.991
		⊿ 0-2 w	0.264 ± 1.697	− 0.243 ± 0.656
42	*Eubacterium ramulus*（*Clostridium* subcluster XIVa）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
43	*Eubacterium* rectale（*Clostridium* subcluster XIVa）	0-week	7.267 ± 7.566	10.512 ± 8.118 #
		2-week	7.570 ± 6.919	9.096 ± 8.553
		⊿ 0-2 w	0.303 ± 6.700	− 1.416 ± 6.476
44	*Eubacterium ventriosum*（*Clostridium* subcluster XIVa）	0-week	0.688 ± 0.930	0.751 ± 0.924
		2-week	0.730 ± 1.162	0.604 ± 0.741
		⊿ 0-2 w	0.041 ± 1.461	− 0.148 ± 1.041
45	*Roseburia intestinalis*（*Clostridium* subcluster XIVa）	0-week	0.043 ± 0.086	0.063 ± 0.110
		2-week	0.033 ± 0.072	0.054 ± 0.139
		⊿ 0-2 w	− 0.010 ± 0.088	− 0.008 ± 0.166
46	*Ruminococcus gnavus*（*Clostridium* subcluster XIVa）	0-week	4.014 ± 6.963	3.247 ± 5.797
		2-week	3.358 ± 6.670	2.743 ± 4.350
		⊿ 0-2 w	− 0.656 ± 2.411	− 0.504 ± 4.233
47	*Ruminococcus obeum*（*Clostridium* subcluster XIVa）	0-week	0.232 ± 0.282	0.231 ± 0.235
		2-week	0.195 ± 0.219	0.269 ± 0.294 #
		⊿ 0-2 w	− 0.037 ± 0.143	0.039 ± 0.256
48	*Ruminococcus torques*（*Clostridium* subcluster XIVa）	0-week	0.324 ± 0.490	0.300 ± 0.469
		2-week	0.314 ± 0.505	0.314 ± 0.443
		⊿ 0-2 w	− 0.010 ± 0.356	0.014 ± 0.263
49	Total of No.37-48（Total of *Clostridium* subcluster XIVa）	0-week	14.503 ± 8.100	17.148 ± 8.478 ‡
		2-week	14.479 ± 7.770	14.764 ± 7.729 †
		⊿ 0-2 w	− 0.023 ± 6.772	− 2.384 ± 7.070
50	*Dialister invisus*（*Clostridium* cluster IX）	0-week	0.287 ± 0.873	0.302 ± 0.794
		2-week	0.304 ± 0.838	0.248 ± 0.808
		⊿ 0-2 w	0.017 ± 0.316	− 0.054 ± 0.407
51	*Megasphaera elsdenii*（*Clostridium* cluster IX）	0-week	0.043 ± 0.188	0.062 ± 0.312
		2-week	0.066 ± 0.268	0.027 ± 0.143
		⊿ 0-2 w	0.023 ± 0.088	− 0.034 ± 0.169
52	*Veillonella ratti*（*Clostridium* cluster IX）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
53	Total of #50-52（Total of *Clostridium* cluster IX）	0-week	0.331 ± 0.925	0.364 ± 0.843
		2-week	0.370 ± 0.934	0.275 ± 0.814
		⊿ 0-2 w	0.039 ± 0.289	− 0.089 ± 0.446
54	*Clostridium bartletti*（*Clostridium* cluster IX）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
55	*Clostridium glycolicum*（*Clostridium* cluster IX）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
56	Total of No.54-55（Total of *Clostridium* cluster IX）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
57	*Clostridium cocleatum*（*Clostridium* cluster XVIII）	0-week	0.000 ± 0.000	0.000 ± 0.000
		2-week	0.000 ± 0.000	0.000 ± 0.000
		⊿ 0-2 w	0.000 ± 0.000	0.000 ± 0.000
58	Total of No.33-57（except for subtotals）	0-week	25.027 ± 10.084	29.243 ± 8.120 #
		2-week	25.407 ± 9.163	24.796 ± 9.531 **
		⊿ 0-2 w	0.380 ± 8.595	− 4.447 ± 8.208 ‡

Unit; %，Values are expressed as the mean ± SD.

† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ indicates a significant difference in comparison to 0-week.（paired t-test）

‡ $p < 0.1$, # $p < 0.05$ indicates a significant difference between two groups.（paired t-test）

Table 6 Results of fecal properties

Item	Unit	Time point	Active (n = 30)	Placebo (n = 30)
Scent of stool	score/1 defecation	0-week	2.75 ± 0.63	2.73 ± 0.61
		2-week	2.85 ± 0.70	2.80 ± 0.63
		⊿ 0-2 w	0.10 ± 0.43	0.07 ± 0.31
Texture of stool	score/1 defecation	0-week	0.92 ± 0.64	0.91 ± 0.88
		2-week	0.69 ± 0.53 †	0.71 ± 0.63
		⊿ 0-2 w	− 0.23 ± 0.59	− 0.19 ± 0.88
Color of stool	score/1 defecation	0-week	1.31 ± 0.55	1.27 ± 0.52
		2-week	1.08 ± 0.49 *	1.19 ± 0.48
		⊿ 0-2 w	− 0.23 ± 0.52	− 0.08 ± 0.51

Values are expressed as the mean ± SD.

† $p < 0.1$, * $p < 0.05$ indicates a significant difference in comparison to 0-week. (Wilcoxon signed- rank test)

colonic motor dysfunction[27], from this, it was suggested that BGL could prevent declining intestinal motor function that was caused by increasing oxidative stress due to poor diet.

In this study, ingestion of food containing BGL suppressed the decrease of *Faecalibacterium prausnitzii*, and decreased *Ruminococcus obeum* and *Clostridium nexile* without loss of *Bifidobacteria* and *Lactobacilli*. Among them, *Faecalibacterium prausnitzii* is especially known to activate intestinal peristaltic movement by producing butyric acid[28]. However, it decreases with aging[29)30)], and is said to be lessened by poor diet and fiber deficiency[31)32)]. Therefore, it was considered that suppression of decrease in *Faecalibacterium prausnitzii* was involved in improvement of bowel movement. In the present study, it was observed that the ingestion of the BGL affected bowel movements habits showing an increase of stool frequency as well as an increase in the volume of stool and maintained *Faecalibacterium prausnitzii* share which was decreased caused by poor diet in the stool. This observation suggests that the BGL can get through to the intestine, adjust the intestinal environment, and improve the bowel movement habits eventually.

Interestingly, BITC, converted from BGL by the intestinal bacteria[33], has selective antibacterial activity against human intestinal bacteria and an inhibitory activity against harmful bacteria (*E. coli*, *C. difficile*, *C. perfringens*), while it has no antibacterial activity against beneficial bacteria (*Bifidobacteria* and *Lactobacilli*)[34)35)]. There was a report that *Faecalibacterium prausnitzii* increased although there was no change in *Lactobacillus* due to ingestion of kale (Brassicaceae) containing the analogous substance of BGL (glucoraphanin)[36]. Therefore, it was suggested that the selective antimicrobial activity of BITC influenced the regulation of the intestinal microbial balance, and as a result, it possibly contributed to improve the bowel movement.

Studies have been conducted for many years on intestinal bacteria targeting bacterial species that could be incubated. Currently the direct sequence focusing in bacteria- specific genes such as: “16S rRNA”, and “16S rDNA” is playing a crucial role in the discovery of bacterial species which were previously unknown[37]. Additionally, in 2003, human gene sequence was wholly deciphered, thanks to the improvement of the precision and speed of the next generation sequencer (NGS), a disorder of the component pattern of intestinal bacteria (dysbiosis) has been linked to various diseases in recent years[3]. The clarification of the role of intestinal bacteria at the species level and factors (age, sex, BMI, lifestyle) correlated with its composition[38] has been advancing, and further studies are necessary.

Secondary Findings

During the course of the present study, the effects of BGL on fecal properties were evaluated. There was no significant difference between the subjects who received the Active and those who were given the Placebo, however the color of the stool changed in a proper way, and the shape of the stool tended to change correctly in the intragroup analysis of the Active. Given the fact that the Placebo did not show any significant differences, it was speculated that BGL contributed to maintaining the intestinal environment so that the fecal properties changed accordingly. Although, it is considered that further studies are necessary.

During the test period, 19 subjects discontinued their participation in the study due to personal circumstances such as business trips. One subject was withdrawn from the analysis due to inadequacy of the records of bowel movement, however the issue was unrelated to the ingestion of the test product. Based upon the diaries of the subjects, we observed no harmful influence on the subjects, which indicated the safety of the ingestion of the test product.

General Information

The Rome criteria for "Functional Constipation Diagnostic" was internationally used to define constipation. In October 2017, the "Chronic constipation clinical practice guideline 2017", an evidence-based clinical practice guideline for adults was published for the first time in Japan. The guideline defined constipation as "a condition in which it is not possible to evacuate adequately and comfortably feces that should be discharged outside the body" [39]. It was thought as a background as follows; many patients suffering from constipation had chief complaints (CC) about their difficulty when defecating rather than about the frequency of stool [40], and chronic constipation may increase with aging. Improvement in bowel movements helps to improve QOL, it is very meaningful to adjust the intestinal environment of individuals without constipation by taking supplements.

Limitations

In this study the screening was performed to choose subjects with the tendency for constipation or the normal level of defecation by means of the "Frequency of bowel movement", and further the principle investigator excluded the subjects with chronic constipation by an interview. It seems that there were many subjects who had no abnormality in the fecal properties such as scent, texture, and color of stool, so that the effect of fecal properties was not evident. A different study design is expected to happen in the future.

5. CONCLUSION

In conclusion, we found out that the effect of 2-week repeated ingestion of BGL-containing food on individuals with the tendency for constipation and individuals with the normal level of defecation showed improvement of bowel movement and intestinal environment. In addition, no safety- related matter occurred during the test period.

CONFLICT OF INTEREST

All parts of this study were funded by Fuji Sangyo Co., Ltd. Shunji Miki and Sei Ozaki are their employees. All authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

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