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Characteristics of Effervescent Tablets of Lactobacilli Supplemented with Chinese Ginseng (*Panax ginseng* C.A. Meyer) and *Polygonatum sibiricum*

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Featured Application: Chinese ginseng (*Panax ginseng* C.A. Meyer) and *Polygonatum sibiricum* are two medicine food homology herbs widely used in many countries, which have many functional benefits. This study demonstrated that it is possible to develop a formulation of lactobacilli effervescent tablets supplemented with Chinese ginseng and *P. sibiricum*, which provides new ideas for the development of functional probiotic products.

Abstract: Development of probiotic products has always been popular in the food industry. Considering the advantages of effervescent tablets, developing probiotic products in effervescent tablet form was conducted in this study. Besides three *Lactobacillus* species, whole root powders of two medicine food homology herbs, Chinese ginseng (*Panax ginseng* C.A. Meyer) and *Polygonatum sibiricum*, were added to the formulation in equal amounts for multiple health care functions. Using the plate counting method, the viability of lactobacilli was measured. After tabletting, lactobacilli viability in tablets containing the two herbs, L-group (20 mg herbs/tablet), M-group (60 mg herbs/tablet), and H-group (100 mg herbs/tablet) was higher than that in the control (containing no herbs). After tablet disintegration, the survival rate of lactobacilli after gastrointestinal fluids treatment was measured; it was higher for the L-group and the H-group than for the control. After incubation with dissolved tablets for 1 h, the lethal rate of *Staphylococcus aureus* and *Escherichia coli* O157:H7 for tablets containing the herbs was lower than that for the control. In the organoleptic assessment test, the L-group and the control were preferred to the M-group and the H-group. During storage at 25 °C for two months, the viability of lactobacilli in tablets containing the herbs was similar to that in the control. In conclusion, the formulation of the L-group has the best characteristics.

Keywords: lactobacilli effervescent tablets; Chinese ginseng; *Polygonatum sibiricum*; lactobacilli viability; antibacterial activity; organoleptic assessment; storage stability

1. Introduction

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2002), such as stimulation of the immune system, maintenance of mucosal integrity, production of important digestive enzymes and others [1]. Most probiotics belong to the genera of *Bifidobacterium* and *Lactobacillus* [2]. *Lactobacillus* species are normal inhabitants of the human gastrointestinal tract, and some of them are generally recognized as safe because of a long history of human consumption [3]. Many pieces of research have led to the conviction that certain strains from the genera *Lactobacillus* can promote health in humans and animals. Among

them, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* have been successfully used in restoring the imbalances associated with inflammatory bowel diseases (IBD) [4,5]. Meanwhile, *Lactobacillus plantarum* has the potential to alleviate aluminium toxicity [6] and inhibit pathogenic fungi [7]. Therefore, these lactobacilli might be useful as supporting therapeutic agents. Considering their probiotic properties, some isolates of these lactobacilli, such as *L. acidophilus* LA1, *L. rhamnosus* GG, and *L. plantarum* Lp01 are already widely used as food supplements and produced on an industrial scale [8].

Due to the beneficial health properties, functional foods have been playing an outstanding role in the food market [9]. As one kind of functional foods, probiotic-supplemented food products are more and more popular for their favorable impact on consumer health. Probiotic products available on the market are mainly fermented dairy products, and fruit drinks and powders containing probiotics can be seen in the marketplace in recent years. However, the most popular forms of probiotic products, liquid, semi-solid, and powder, require more space for transportation and storage, which may increase the price of end products. To overcome such problems, compaction of powders containing probiotics in the shape of tablets is a more suitable strategy [10]. In addition to reducing the bulk volume of other forms, products in tablet form are extremely stable, surpassing the stability of liquid form [11]; thus they lead to prolonged shelf life.

At present, the demand for ready-to-eat food and drinks is increasing day by day. Ready-to-drink effervescent tablets can meet consumer demands. Meanwhile, effervescent tablets containing probiotics can be added to any liquid product, which would provide a pleasant taste and facilitate the daily ingestion of probiotics. They are especially suitable for children, the elderly, and people who have difficulty in swallowing pills. Additionally, carbon dioxide gas released during effervescent tablet dissolving could stabilize the gastric mucosa and promote absorption, resulting in benefits to consumer health [12]. Therefore, water-soluble effervescent tablets are a good choice for probiotic-supplemented food products.

To our knowledge, effervescent tablets containing probiotics are usually used for the prevention of vaginal infections [13,14], but little research has been done on the development of effervescent tablets supplemented with probiotics for application in the food industry. Using *L. acidophilus* and *Saccharomyces boulardii* as probiotics supplements, Nagashima, Pansiera, Baracat and Gómez [12] had developed probiotic effervescent tablets for ready-to-drink. Considering the health needs of consumers and the good properties of probiotics, more studies should be conducted to develop a wider variety of effervescent tablet products supplemented with probiotics, especially with multiple health care functions.

Chinese ginseng (*Panax ginseng* C.A. Meyer) is the medicine food homology plant, which has been used for over 2000 years as a medicine in China and is popularly used in more than 35 countries as food, a health supplement, and a natural remedy [15]. Ginseng possesses many beneficial health properties, such as the alleviation of menopause symptoms and some neurological disorders, regulation of cardiovascular disease and hyperglycemia in type 2 diabetes, and improvement of the immune function [16]. *Polygonatum sibiricum* is another traditional herbal medicine and foodstuff widely used in China, Korea, and Japan for hundreds of years [17]. The dried root of *P. sibiricum* shows anti-inflammatory, immunoregulatory, and anti-diabetic activities [18]. Hence, the present work aimed to develop effervescent tablet products containing both probiotic lactobacilli and Chinese ginseng and *P. sibiricum* for more health care functions. Characteristics of the effervescent tablet products were investigated, especially the effect of Chinese ginseng and *P. sibiricum* on the properties of lactobacilli effervescent tablets.

2. Materials and Methods

2.1. Strains

Three lyophilized strains of lactobacilli obtained from Clerici Sacco Co. (Italy) were used in the preparation of effervescent tablets. They were *L. acidophilus* LA3, *L. rhamnosus* IMC501,

and *L. plantarum* BG112. These strains are often used in functional foods as probiotic strains and dietary supplements [19–21]. In the antibacterial experiment, *Staphylococcus aureus* NCTC 8325-4 and *Escherichia coli* O157:H7 NCTC 12900 were used. *S. aureus* NCTC 8325-4 belongs to the gram-positive bacterium, which was kindly donated by Professor Baolin Sun working in the University of Science and Technology of China. *E. coli* O157:H7 NCTC 12900, one of the gram-negative bacteria, was obtained from the National Culture Type Collection (Colindale, London, UK).

2.2. Preparation of Effervescent Tablets

In order to maintain high probiotic activity, probiotics used in product production should possess high cell viability. According to the viability of three kinds of *Lactobacillus* in lyophilized powder under the same culture condition as in our preliminary experiment, the proportion of three lactobacilli strains in the mixed lactobacilli powder for effervescent tablet preparation was achieved, which was 5:4:1 for *L. plantarum*: *L. rhamnosus*: *L. acidophilus*. As citric acid and sodium bicarbonate are the common effervescent agents used in pharmaceutical and nutraceutical industries to produce fast dissolving effervescent tablets, the two agents were added to the effervescent tablets in this study. Additionally, the formulation of effervescent tablets contained povidone as a tablet binder and polyethylene glycol 6000 (PEG6000) as a lubricating agent. To determine the effect of Chinese ginseng and *P. sibiricum* on the properties of lactobacilli effervescent tablets, effervescent tablets containing different contents of Chinese ginseng and *P. sibiricum* were prepared. Whole root powders of the two herbs were obtained from Changchun Huacheng Biological Co., Ltd. (Changchun, China). The composition formulas for the lactobacilli effervescent tablets are given in Table 1. Each ingredient for tablet preparation was calibrated through an 80-mesh screen, and then the weighed ingredients were mixed thoroughly according to the formulation. A single punch tabletting machine (Hebi Innovative Instruments Co., Ltd., Henan, China) equipped with a 12-mm flat-faced round punch was used for the preparation of the effervescent tablets.

Table 1. Formulations of lactobacilli effervescent tablets supplemented with different contents of Chinese ginseng and *Polygonatum sibiricum*.

Ingredients	Tablets Formulations (Quantity in mg/Tablet)			
	Control	L-Group	M-Group	H-Group
Lactobacilli mixed powder	150	150	150	150
Citric acid	350	350	350	350
Sodium bicarbonate	350	350	350	350
Povidone	30	30	30	30
PEG6000	20	20	20	20
Chinese ginseng powder	0	10	30	50
Polygonatum sibiricum powder	0	10	30	50

2.3. Measurement of Weight and Thickness of Tablets

For each kind of tablet, ten tablets were individually and randomly weighed on an analytical balance (OHAUS International Trade (Shanghai) Co., Ltd., Shanghai, China) to determine their weight, and their thickness was also measured by a ruler.

2.4. Determination of Disintegration Time

According to the method for measurement of disintegration time of effervescent tablets in Chinese Pharmacopoeia (2015 Edition), the disintegration time of the effervescent tablets prepared in this study was tested. For each kind of effervescent tablet, the disintegration time of six tablets was individually determined in 200 mL of distilled water at 20 ± 5 °C. Disintegration time within 5 min is considered acceptable.

2.5. Change of Viability of Lactobacilli Cells after Tabletting and Tablet Disintegration

In order to determine survival rates of lactobacilli cells after the tablets preparation, the number of viable lactobacilli cells in the mixed lactobacilli powder before tabletting and in the tablets was measured using the pouring plate method. Firstly, the mixed lactobacilli powder or the tablets were dissolved in sterile distilled water. Then, the dissolved sample was ten-fold diluted with a 0.90% NaCl solution, and pour-plated on de Man-Rogosa-Sharpe (MRS) agar (Oxoid Ltd., Basingstoke, Hants, UK). Each dilution sample was measured in triplicate. After incubation at 37 °C for 48 h, the number of colonies was counted. Two independent experiments were performed. The survival rate of lactobacilli cells after tablet preparation equals to the ratio of viable cells in the tablets to those in the mixed lactobacilli powder.

Furthermore, to investigate the viability change of lactobacilli cells after tablet disintegration, the remaining tablet solution after counting was placed at 25 °C for 48 h, then the number of viable lactobacilli cells was measured using the pouring plate method. The survival rate of lactobacilli cells after tablets disintegration was calculated by the ratio of viable cells in tablet solution after disintegration for 48 h to those for 0 h.

2.6. Effect of Gastrointestinal Fluids on the Viability of Lactobacilli Cells

Firstly, one effervescent tablet was dissolved in 15 mL of sterile distilled water. Then, 0.1 mL and 1.0 mL samples from the same solution were added to 9.9 mL and 9.0 mL of sterile simulated gastric fluid (0.045 M HCl, 1% pepsin, pH 1.7), respectively. After incubation at 37 °C for 30 min, 1.0 mL aliquot was removed and added to 9.0 mL of sterile simulated intestinal fluid (0.05 M KH_2PO_4, pH 6.8, added with 0.6% of bile salts), which was further incubated at 37 °C for 150 min. The number of viable lactobacilli cells before and after gastrointestinal fluids treatment was determined using the pouring plate method. Meanwhile, the survival rate after gastric or enteric fluid treatment was calculated respectively, which equals the ratio of viable cells after treatment to those before treatment. Two independent experiments were performed.

2.7. Analysis of the Antibacterial Activity of the Tablets

Antibacterial studies were performed against *S. aureus* and *E. coli* O157:H7. After activation from the stocked strain, cultures of *S. aureus* or *E. coli* O157:H7 were inoculated into sterile distilled water, and the initial cell counts were measured by the pouring plate method using tryptic soy agar (TSA). Each sample was measured in duplicate. Then one effervescent tablet was added to 15 mL of the bacterial solution, and the dissolution time was recorded. After incubation for one hour (including dissolution time) at room temperature, the number of *S. aureus* or *E. coli* O157:H7 was determined by the staph express count plate or *E. coli*/Coliform count plate of 3M Petrifilm^{TM} (3M Center, St. Paul, MN, USA). Each sample was measured in duplicate. The testing slips were incubated at 37 °C for 48 h. Two independent experiments were performed. The antibacterial activity of the tablets was assessed by the following equation:

$$\text{antibacterial ratio} = \left(1 - \frac{\text{Cell counts of bacteria after incubation with tablets for one hour}}{\text{Initial cell counts of bacteria}}\right) \times 100\%$$

Meanwhile, the pH of the solution was measured, including pH before adding tablets, pH at the end of dissolving tablets, and pH after incubation with tablets for one hour. The pH measurement was performed for three independent experiments.

2.8. Organoleptic Assessment

Twenty panelists (10 males and 10 females) aged between 22 and 25 years participated in the organoleptic assessment. The panelists were selected from students at the College of Food Science and Engineering at Jilin University, China. The study was reviewed and approved by the college of Food

Science and Engineering at Jilin University. All participants were familiar with basic organoleptic assessment techniques, and all of them agreed to participate in the test. Before the organoleptic assessment, the panelists participated in briefing sessions to familiarize themselves with the specific vocabulary used to describe the effervescent tablet solution attributes and with the evaluation scales.

Each kind of sample solution was prepared by adding one tablet into 200 mL distilled water at room temperature. After disintegration, the panelists were presented with 20 mL of each sample in see-through polypropylene cups. Different samples were labeled with random numbers and presented to each panelist in random order. Between samples evaluation, panelists were asked to cleanse their palate with water. In addition, panelists were required to have a break before starting the next evaluation [22].

Attributes of appearance, color, aroma, flavor, and overall liking were evaluated in a 9-point hedonic scale ranging from 1 ("dislike extremely") to 9 ("like extremely"). Afterwards, the products were ranked and evaluated by the Friedman test [23] to verify differences between samples.

2.9. Stability of the Tablets during Storage

In order to study the stability of tablets during storage, the tablets were stored individually in vacuum packages (Sealed Air Co., Charlotte, NC, USA), which are transparent multi-layer co-extruded cross-linked vacuum shrinkable bags with high oxygen barrier, high sealing strength, and good wear resistance and shrinkage properties, and then incubated at 25 °C for two months. At a certain storage time (0, 15, 30, 45 and 60 days), one tablet of each kind of tablets was dissolved in 15 mL sterile distilled water, and then the number of lactobacilli in the solution was measured in triplicate using MRS agar by the pouring plate method. Two independent experiments were performed.

2.10. Statistical Analysis

One-way analysis of variance (ANOVA) and student's test were used to analyze possible statistically significant differences among the samples. The significance level was 0.05. All the analyses were performed using IBM SPSS Statistics 22 (Chicago, IL, USA). The figure was produced by Microcal Origin 9.2 (Northampton, MA, USA).

3. Results and Discussion

3.1. Physical Properties of the Tablets

Round, flat disc-shaped tablets were obtained in this study. As powders of Chinese ginseng and *P. sibiricum* are yellow, and other raw materials are white powders, the color of tablets without Chinese ginseng and *P. sibiricum* (Control) was white, while tablets supplemented with the two herbs showed pale yellow. Besides the color, weight and thickness of tablets prepared by different formulations were different. As shown in Table 2, the mean weight of the control was 0.82 g per tablet, which was increased with an increase in the content of the two herbs. Except for the H-group (100 mg herbs/tablet), the thickness of the other three tablets showed similar results (Table 2). After adding the tablets to distilled water, the effervescence was mediated by the presence of citric acid and sodium bicarbonate. The disintegration time of the control was short, at about 3.19 min. Interestingly, this parameter was influenced by the presence of the herbs, showing prolonged disintegration significantly with increasing content of the two herbs ($p < 0.05$). It was 4.48, 6.92, and 9.70 min for the L-group (20 mg herbs/tablet), the M-group (60 mg herbs/tablet), and the H-group, respectively (Table 2). The result indicated that the presence of the two herbs has a slow-releasing effect on the disintegration of effervescent tablets. A disintegration time within 5 min for oral effervescent tablets is considered acceptable in the pharmacopoeia of several countries. The disintegration time depends on the disintegrating solvent, solvent temperature and solvent volume, thus suitable disintegrating conditions should be studied for the M-group and H-group tablets.

Table 2. Weight, thickness and disintegration time of the lactobacilli effervescent tablets supplemented with different contents of Chinese ginseng and *Polygonatum sibiricum.*

Tablets	Weight (g)	Thickness (mm)	Disintegration Time (min)
Control	0.82 ± 0.02 [a]	7.05 ± 0.37 [a]	3.19 ± 0.41 [a]
L-group	0.85 ± 0.03 [b]	6.90 ± 0.39 [a]	4.48 ± 0.31 [b]
M-group	0.88 ± 0.02 [c]	7.40 ± 0.61 [a]	6.92 ± 0.33 [c]
H-group	0.92 ± 0.02 [d]	8.15 ± 0.24 [b]	9.70 ± 0.42 [d]

All data show the means ± SD. Different letters in the same column indicate a significant difference.

3.2. Change of Viability of Lactobacilli after Tabletting and Tablet Disintegration

In order to achieve benefits from probiotics, probiotic products must contain at least 10^6 CFU (Colony Forming Unit)/g or be eaten in sufficient amounts to yield a daily intake of 10^8 CFU [24]. Therefore, the number of viable lactobacilli cells in tablets was investigated. As shown in Table 3, at least 1.55 ± 0.80 billion viable cells per tablet were found after disintegration, which meets the intake requirement for probiotics. However, compared with the mixed lactobacilli powder before tabletting, the number of viable cells in tablets decreased remarkably for four kinds of tablets. Nagashima, Pansiera, Baracat and Gómez [12] had also found that there was up to 2.3 logarithmic cycles reduction in the number of viable *L. acidophilus* in effervescent probiotics tablets after tabletting. Chan and Zhang [25] had observed that cellular viability decrease was related to the applied compression force in tabletting. Under mechanical stress, some cells cannot tolerate such compression, which caused damages to the cell walls and membranes or even loss of viability [12]. But it is worth noting that the survival rate of lactobacilli cells was higher for tablets containing the herbs than that for the control, and the survival rate enhanced significantly with the increase of the herbs content in tablets ($p < 0.05$) (Table 3). This result demonstrated that Chinese ginseng and *P. sibiricum* can protect the viability of lactobacilli cells in the tablets. Perhaps some active ingredients in the herbs, such as polysaccharides, might maintain the viability of the lactobacilli. As we know, prebiotics supply a fermentable carbohydrate for probiotic bacteria in the colon, stimulating the growth and activity [26]. Thus, we further speculated that the two herbs may have similar functions with prebiotics, which would protect or promote the viability of beneficial bacteria after consumption. Therefore, the viability of lactobacilli cells after gastrointestinal juices treatment for four kinds of tablets was investigated, which will be discussed in a later part in this study.

Table 3. Viability of lactobacilli cells in the mixed lactobacilli powder before tabletting and in different kinds of lactobacilli effervescent tablets.

Samples	Number of Viable Cells	The Survival Rate after Tabletting
Mixed lactobacilli powder	$(7.14 \pm 0.52) \times 10^{10}$ CFU/g powder	
Control tablet	[#] $(1.55 \pm 0.80) \times 10^9$ CFU/Tablet [a]	14.50%
L-group tablet	$(5.18 \pm 3.71) \times 10^9$ CFU/Tablet [b]	48.37%
M-group tablet	$(7.00 \pm 1.14) \times 10^9$ CFU/Tablet [b,c]	65.36%
H-group tablet	$(9.03 \pm 1.77) \times 10^9$ CFU/Tablet [c]	84.31%

All data show the means ± SD. [#] ANOVA was performed among different kinds of effervescent tablets. Different letters in the same column indicate a significant difference.

What is the change of lactobacilli viability after tablet disintegration? Do the herbs protect viability or stimulate the growth of lactobacilli after tablet disintegration? Surprisingly, after disintegration for 48 h, the number of lactobacilli cells for the control tablet increased more than 2-fold, while the survival rate for tablets containing the herbs decreased by 10.5%–23.75% (Table 4). In fact, besides polysaccharides, saponins are another main active component in ginseng and *P. sibiricum* which have antimicrobial activity [27–29]. Battinelli, Mascellino, Martino, Lu and Mazzanti [29] found that a total extract of ginseng had lower antimicrobial activity than some pure ginsenosides (saponins in ginseng),

and ascribed this lack of antimicrobial activity of the whole plant to possible antagonist effects between ginsenosides and other ginseng components. Considering the protective effect of the herbs on the viability of lactobacilli after tabletting, the changes of the viability of lactobacilli after tabletting and tablet disintegration for tablets containing the herbs may be due to possible antagonistic effect between saponins and other components in the herbs.

Table 4. The survival rate of lactobacilli cells after tablet disintegration for 48 h.

Tablets	The Survival Rate after Tablet Disintegration for 48 h
Control	216.61% ± 0.86 [b]
L-group	76.25% ± 0.13 [a]
M-group	89.50% ± 0.21 [a]
H-group	77.62% ± 0.16 [a]

All data show the means ± SD. Different letters in the same column indicate a significant difference.

3.3. Effect of Gastrointestinal Fluids on the Viability of Lactobacilli Cells

As shown in Table 5, the survival rate of lactobacilli cells after gastric juice treatment for each kind of tablets was remarkably different with the different initial amounts of tablet solution inoculated into gastric juice. It was increased 23-, 350-, 186- or 482-fold for tablets of control, L-group, M-group, or H-group, respectively, when the initial inoculum increased from 0.1 mL to 1.0 mL. With a 10-fold increment in the initial inoculum, the survival rate of lactobacilli cells after gastric juice treatment increased more than 10-fold for all the kinds of tablets, which demonstrated that more intake of the probiotic product would greatly increase the levels of probiotics reaching the intestine. Meanwhile, it can be seen that the effervescent tablets supplemented with the herbs have a higher survival rate of lactobacilli cells after gastric fluid treatment than the control at 1.0 mL initial inoculum, but this phenomenon cannot be found at 0.1 mL initial inoculum (Table 5). This result indicates that the herbs supplemented in tablets have a protective effect on the viability of lactobacilli during gastric fluid treatment, and this protective effect of the herbs is dose-dependent. This protective effect caused by the herbs should also be a comprehensive effect of antimicrobial activity and probiotic activity derived from components in the herbs. However, in the gastric juice environment, antimicrobial components in the herbs may not play their role effectively, and/or probiotic activity of components may be improved, thus the protective effect is prominent.

Table 5. The survival rate of lactobacilli cells after contact with gastric juice for 30 min followed by exposure to enteric juice for 150 min for different kinds of lactobacilli effervescent tablets.

Tablets	The Initial Amount of Tablet Solution Inoculated into Gastric Juice	Survival Rate (%)		
		Gastric Juice Treatment (N_g/N_0) [#]	Enteric Juice Treatment (N_e/N_g) [#]	Enteric Juice Treatment (N_e/N_0) [#]
Control	0.1 mL	0.0369	0.0000	0.0000
L-group	0.1 mL	0.0063	0.3992	0.0000
M-group	0.1 mL	0.0077	0.0000	0.0000
H-group	0.1 mL	0.0155	0.0000	0.0000
Control	1.0 mL	0.8660	0.1167	0.0009
L-group	1.0 mL	2.2162	0.3444	0.0076
M-group	1.0 mL	1.4232	0.0442	0.0006
H-group	1.0 mL	7.4842	0.3239	0.0294

[#] N_0 is the number of viable lactobacilli cells before gastric fluid treatment (CFU/Tablet). N_g is the number of viable lactobacilli cells after gastric fluid treatment (CFU/Tablet). N_e is the number of viable lactobacilli cells after enteric fluid treatment (CFU/Tablet).

After enteric juice treatment, there were no viable lactobacilli cells at 0.1 mL initial inoculum for three kinds of tablets, while viable cells could be examined in all the kinds of tablets at 1.0 mL initial inoculum (Table 5). This result also confirmed that more intake of the product would significantly

increase the levels of probiotics in the intestine. However, compared with the control, the survival rate of lactobacilli cells after intestinal juice treatment only increased 2.95-, 0.13- and 2.77-fold for L-group, M-group, and H-group, respectively. Meanwhile, there was no dose-dependent protective effect of the herbs on the viability of lactobacilli cells after enteric juice treatment when compared to the gastric fluid treatment. Nagashima, Pansiera, Baracat and Gómez [12] had also found that the number of *L. acidophilus* decreased significantly after effervescent probiotics products contact with artificial enteric juice; they ascribed this decline to the sharp change in the pH from 2.0 in the gastric juice to 6.8 in the enteric juice, and the presence of bile salts. Therefore, the presence of bile salts in enteric juice and the pH variation from 1.7 to 6.8 might greatly destroy the viability of lactobacilli and thus weaken the protective effect of the herbs in this study. Additionally, an increase of antimicrobial activity and/or a decrease of probiotic activity of components in the herbs in the enteric juice environment may be another reason for the reduced protective effect of the herbs.

Generally, after gastrointestinal fluids treatment, the survival rates of lactobacilli for the L-group (0.0076%) and the H-group (0.0294%) were both higher than that for the control (0.0009%), while it was similar between the M-group (0.0006%) and the control. It is confirmed that the herbs supplemented in the lactobacilli effervescent tablets have a certain protective effect on the viability of lactobacilli during gastrointestinal fluids treatment, but for the better protective effect the optimal concentration of the herbs in probiotic products should be further investigated.

3.4. Antibacterial Activity of the Tablets

The goal in administering probiotic products is to induce a balanced enteric microbiota, which will have a favorable impact on consumer health. Enteric microbiota include both beneficial and harmful microflora. As mentioned above, the tablets supplemented with Chinese ginseng and *P. sibiricum* have a certain favorable effect on the activity of some lactobacilli. In order to investigate the antibacterial activity of the lactobacilli effervescent tablets, an in vitro bacteriostatic experiment against *S. aureus* and *E. coli* O157:H7, two common pathogens in clinical infection and food contamination, was conducted. After incubation with the control tablet for one hour, about 95.11% and 97.86% of *S. aureus* and *E. coli* O157:H7 were killed, respectively (Table 6). While the antibacterial ratio against both bacteria was decreased significantly with an increase in the content of the two herbs in the tablets ($p < 0.05$), there was still a high antibacterial ratio for these tablets, which was more than 78% for the H-group. Because the treatment time of the antibacterial experiment was relatively short, which was 1 h, we considered that the disintegration progress of tablets may be an important factor affecting the antibacterial activity. As shown in Table 6, the dissolution time of the tablets was prolonged with increasing content of the two herbs ($p < 0.05$), which is opposite to the change trend of antibacterial activity. The effervescence mediated by the citric acid and sodium bicarbonate ensures a rapid and complete distribution of the active ingredients in products to solutions [14], and can rapidly create an anaerobic microenvironment by released CO_2 [30], which can also decrease pH of the solution (Table 6). These features are harmful to bacteria which cannot withstand the environment. As the dissolution time of the tablets was prolonged with increased content of the two herbs (Table 6), this would decrease release rates of active ingredients and CO_2 with an increase in the content of the herbs, which may lower the antibacterial activity of the tablets with increased content of the herbs. Additionally, under the condition of the antibacterial experiment in this study, antagonist effects between antimicrobial and probiotic components of the herbs may lead to weak antibacterial activity of the tablets containing the herbs in a dose-dependent manner. Therefore, the antibacterial activity was highest for the control, which decreased significantly with an increase in the content of the two herbs in tablets under the condition of the antibacterial experiment in this study (Table 6). According to the previous results on the change of viability of lactobacilli after tablet disintegration for 48 h (Table 4), we deduced that the antibacterial activities of the four kinds of tablets against *S. aureus* and *E. coli* O157:H7 would be different when prolonged the treatment time.

Table 6. Antibacterial activity of different kinds of lactobacilli effervescent tablets against *Staphylococcus aureus* and *Escherichia coli* O157:H7 for one-hour incubation.

Tablets	Antibacterial Ratio against *S. aureus*	Antibacterial Ratio against *E. coli* O157:H7	Dissolution Time (min)	pH of Solution		
				Without Tablets	End of Tablets Dissolving	Incubation for 1 h
Control	95.11% ± 0.01 ^{d,A}	97.86% ± 0.01 ^{c,B}	1.99 ± 0.36 ^{a}	6.38 ± 0.09 $^{\beta}$	5.61 ± 0.03 $^{\alpha}$	5.71 ± 0.08 $^{\alpha}$
L-group	90.00% ± 0.01 ^{c,A}	97.39% ± 0.00 ^{c,B}	2.28 ± 0.26 ^{a}	6.42 ± 0.06 $^{\beta}$	5.56 ± 0.08 $^{\alpha}$	5.58 ± 0.08 $^{\alpha}$
M-group	83.16% ± 0.02 ^{b,A}	90.96% ± 0.02 ^{b,B}	3.27 ± 0.28 ^{b}	6.59 ± 0.05 $^{\beta}$	5.57 ± 0.04 $^{\alpha}$	5.60 ± 0.03 $^{\alpha}$
H-group	78.27% ± 0.03 ^{a,A}	83.87% ± 0.04 ^{a,B}	4.45 ± 0.04 ^{c}	6.72 ± 0.11 $^{\beta}$	5.52 ± 0.03 $^{\alpha}$	5.53 ± 0.03 $^{\alpha}$

All data show the means ± SD. Different lowercase English letters in the same column indicate a significant difference; different uppercase English letters or lowercase Greek letters in the same row indicate a significant difference, respectively.

Meanwhile, the antibacterial activity of each kind of tablet was significantly higher against *E. coli* O157:H7 than against *S. aureus* ($p < 0.05$) (Table 6). Xue, Yang, Zhao, Hou, Zhang, Zhang and Ren [28] had found that ginsenosides cause bacterial death by directly destroying the membrane system. As we know, the cell wall of *S. aureus* is thicker than that of *E. coli* O157:H7, which will reduce the speed and content of CO_2 and active ingredients (such as saponins) reaching the cell membrane, causing higher antibacterial activity against *E. coli* O157:H7 than against *S. aureus* for each kind of tablet.

3.5. Organoleptic Assessment

An organoleptic assessment was performed to measure the acceptability of the four kinds of effervescent tablets. As shown in Table 7, except for appearance and overall liking attributes, there was no significant difference among different kinds of tablets. In respect of the appearance of the tablet solution, panelists evaluated the L-group as largely like (7.30 ± 1.17), while the H-group was considered slightly appreciated (5.90 ± 1.86). After disintegration, a small amount of light-yellow foam was floating on the solution of the H-group, resulting in a lower appearance score for it. The overall liking classified the H-group as slightly like (5.30 ± 1.56) and the L-group and the control as moderately like (6.45 ± 1.43 and 6.05 ± 1.39, respectively), while the evaluation for the M-group (5.70 ± 1.45) was between them. Besides the appearance attribute, the flavor was also a main reason for a lower overall liking score for the H-group solution, although there was no significant difference in the flavor attribute between the four kinds of tablets ($p > 0.05$). As can be seen from Table 7, the lowest score of flavor attribute was found for H-group solution (5.70 ± 1.34), as most panelists felt relatively high bitterness in the H-group solution. Panelists participated in the organoleptic assessment aged between 22 and 25 years, do not like consuming bitter foods. Perhaps these effervescent tablets may be evaluated differently if older people participate in the organoleptic assessment. According to the preference ranking test results (Table 8), the L-group received the highest value in the sum of the orders (59), but its preference did not present statistical difference ($p > 0.05$) for the control and the M-group. The H-group was the less preferred along with the M-group, reinforcing the results obtained in the organoleptic assessment.

Table 7. Organoleptic assessment of the lactobacilli effervescent tablets supplemented with different contents of Chinese ginseng and *Polygonatum sibiricum.*

Tablets	Appearance	Color	Aroma	Flavor	Overall Liking
Control	7.10 ± 1.41 ^{a,b}	7.25 ± 0.97 ^{a}	6.15 ± 1.31 ^{a}	6.30 ± 1.49 ^{a}	6.05 ± 1.39 ^{a}
L-group	7.30 ± 1.17 ^{b}	7.20 ± 0.95 ^{a}	6.40 ± 1.39 ^{a}	6.40 ± 1.43 ^{a}	6.45 ± 1.43 ^{a,b}
M-group	6.20 ± 1.58 ^{a,b}	6.75 ± 1.37 ^{a}	6.35 ± 1.22 ^{a}	6.10 ± 1.71 ^{a}	5.70 ± 1.45 ^{a}
H-group	5.90 ± 1.86 ^{a}	6.55 ± 1.54 ^{a}	6.40 ± 1.23 ^{a}	5.70 ± 1.34 ^{a}	5.30 ± 1.56 ^{a}

All data show the means ± SD. Different letters in the same column indicate a significant difference.

Table 8. Results of the preference ranking test of the lactobacilli effervescent tablets supplemented with different contents of Chinese ginseng and *Polygonatum sibiricum*.

Tablets	Control	L-Group	M-Group	H-Group
Control	-	1 ^{ns}	10 ^{ns}	23 *
L-group		-	11 ^{ns}	24 *
M-group			-	13 ^{ns}
H-group				-

* Significant critical difference (5%) minimum of 21, for four samples and 20 panelists, according to the Friedman test. ^{ns} non-significant.

3.6. Stability of the Tablets during Storage

Figure 1 shows the survival of lactobacilli in different kinds of lactobacilli effervescent tablets after storage at 25 °C for 60 days. After storage for 15 days, the number of lactobacilli cells was reduced by 1.20, 1.79, 2.08, and 2.35 logarithmic cycles for the control, L-group, M-group, and H-group, respectively. This result demonstrated that the viability of lactobacilli decreased significantly with the increase of the content of the herbs in tablets in the early stage of storage (0–15 days). Nevertheless, in the later stage of storage (15–60 days), the viability of lactobacilli in the tablets supplemented with the herbs was similar to that in the control (Figure 1). Considering the diversity of drug-resistance ability of microorganism cells to antimicrobial substances, we speculated that some sensitive cells in the tablets may be inactivated by the herbs in a dose-dependent manner in the early stage of storage, while the cells that can tolerate the herbs could maintain their viability throughout the storage period. Nagashima, Pansiera, Baracat and Gómez [12] had also reported that the number of *L. acidophilus* in effervescent probiotics tablets decreased from 5.18 to 2.91 logarithmic cycles after storage for 60 days at 25 °C. In this study, the number of viable lactobacilli cells was about 5.80 logarithmic cycles in all kinds of tablets at the end of storage (Figure 1). In order to obtain more probiotic properties, it should be considered to add protectants for lactobacilli viability to the formula.

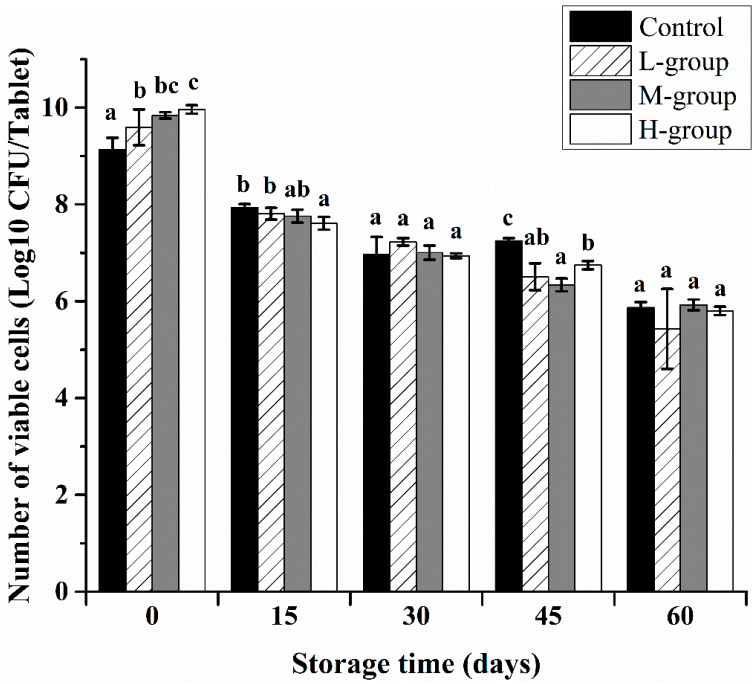

Figure 1. Viability of lactobacilli in different kinds of lactobacilli effervescent tablets stored for 0, 15, 30, 45 and 60 days at 25 °C. All data show the means ± SD. At the same storage period, different letters indicate a significant difference.

4. Conclusions

Our findings demonstrate that it is possible to develop an optimal formulation of lactobacilli effervescent tablets supplemented with Chinese ginseng and *P. sibiricum*, combining functional benefits of lactobacilli and both herbs. In terms of form, the effervescent tablet product exceeds the stability of the liquid form, thereby prolonging the shelf life. Compared with the capsule product, the effervescent tablet has the advantages of simple processing, rapid disintegration, dissolving in water, and easy to take. The effervescent tablet is more suitable for children, the elderly, and people who have difficulty in swallowing pills. According to the results in this study, the formulation of the L-group (60 mg herbs/tablet) would be the best choice for industrial production, which can effectively protect the viability of lactobacilli after tabletting and gastrointestinal juices treatment, together with the high antibacterial ability and the preferred organoleptic characteristics. Meanwhile, the L-group was relatively stable during storage. For a better probiotic effect of the L-group, consumers should drink it in time after disintegration, which would be conducive to retaining higher viability of lactobacilli reaching the intestine and help to create better antibacterial activity in the intestine, leading to a more beneficial enteric microbiota. The main purpose of this study was to investigate the effect of Chinese ginseng and *P. sibiricum* on the properties of effervescent tablets, thus no sweetener was added to the formulations of the lactobacilli effervescent tablets. Considering the younger consumer perspective, manufacturers can add some beneficial sweeteners, such as the prebiotic fiber inulin, to the formula. Additionally, an in vivo experiment should be studied for understanding more health care functions of the lactobacilli effervescent tablets supplemented with Chinese ginseng and *P. sibiricum*.

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