Original Article

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Decreased sugar concentration in vegetable and fruit juices by growth of functional lactic acid bacteria

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Summary ***Leuconostoc carnosum* #7-2, *L. gelidum* #4-2, and *L. mesenteroides* 8/11-3, which were isolated from fermented plant foods, are lactic acid bacteria. We previously reported that these bacteria are functional lactic acid bacteria whose innate immunity-stimulating activities are high based on a silkworm muscle contraction assay. The concentrations of these three lactic acid bacteria increased to more than 1 × 10^6 colony forming units (cfu)/mL in various vegetable and fruit juices when the pH values were appropriately adjusted. As the bacteria grew in the vegetable and fruit juices, the pH decreased and the concentrations of total sugars and glucose also decreased. These findings suggest that these functional lactic acid bacteria can be used to produce vegetable and fruit juices with reduced sugar levels, which is expected to be beneficial for human health.**

Keywords: Juice, lactic acid bacteria, sugar concentration

1. Introduction

Vegetable and fruit juices are commonly ingested beverages. These juices contain abundant sugars, including sucrose, glucose, and fructose, and their ingestion may lead to excessive intake of carbohydrates. Reducing the sugar levels in vegetable and fruit juices may help to decrease the risk of lifestyle-related diseases such as diabetes and obesity resulting from excessive carbohydrate ingestion. Unlike the methods that are used to decrease the sugar content of carbonated drinks, sports drinks, and coffee drinks, methods that effectively remove sugars from vegetable and fruit juices without affecting the taste of the juice are limited.

Lactic acid bacteria are used to ferment foods, such as yogurt and pickles. We have studied functional lactic acid bacteria with innate immunity-stimulating activity and postprandial hyperglycemia inhibitory activity (*1,2*). The use of functional lactic acid bacteria for manufacturing fermented food is thought to contribute to maintaining human health. We demonstrated that stimulating the silkworm immune system induces the production of a cytokine called paralytic peptide, whose pharmacologic activities include muscle contraction (*3*). Lactic acid bacteria strains *Leuconostoc carnosum* #7-2, *L. gelidum* #4-2, and *L. mesenteroides* 8/11-3, which were previously isolated from kimchi and rice bran (fermented plant foods) have high innate immunity-stimulating activities as determined using the silkworm muscle contraction assay.

In this article, we describe that sugar concentrations in juices can be reduced by these lactic acid bacteria. Vegetable and fruit juices, in which functional lactic acid bacteria grow, are expected to be useful for promoting health.

2. Materials and Methods

2.1. *Lactic acid bacteria*

The lactic acid bacteria used in this study are listed in Table 1.

2.2. *Growth of lactic acid bacteria in vegetable and fruit juices*

Lactic acid bacteria were precultured anaerobically

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in deMan, Rogosa and Sharpe (MRS) liquid media (Becton, Dickinson and Company, MD, USA) at 30°C. Vegetable and fruit juices (50 mL) were added to 50 µL to 500 µL of precultured lactic acid bacteria, and further cultured at 30°C for 24 to 94 h. The number of viable cells was calculated by counting the number of colonies that formed on MRS agar plates spread with diluted samples and anaerobically cultured at 30°C. The pH of the culture was maintained between 6 and 7 by adding a 10N NaOH solution.

Glycerol stocks of lactic acid bacteria were prepared and stored at -80°C. Specifically, a single colony was isolated from MRS agar (Becton, Dickinson and Company) containing 0.5% calcium carbonate (Wako Pure Chemical Industries, Osaka, Japan) that was spread with lactic acid bacteria and anaerobically cultured at 30°C. The bacterial colony was picked up and inoculated in 15 mL of MRS media, and anaerobically cultured at 30°C. Bacterial pellets were collected after centrifugation. The pellet was suspended into 2 mL of 0.9% NaCl (Wako Pure Chemical Industries), and mixed well with same volume of 80% glycerol (Wako Pure Chemical Industries). The glycerol stocks were stored at -80°C.

Table 1. Lactic acid bacteria used in this study

Strains	Origins	Species
#7-2	Kimchi	*Leuconostoc carnosum*
#4-2	Rice bran	*Leuconostoc gelidum*
8/11-3	Kimchi	*Leuconostoc mesenteroides*

2.3. *Determination of sugar concentrations in vegetable and fruit juices*

Total sugar concentration in the juices was determined by the phenol-sulfuric acid method according to the following procedure: Each juice sample (100 µL) was centrifuged at 8,000 rpm for 10 min. Phenol (5%; Wako Pure Chemical Industries) was added to the sample and vortexed vigorously for 5 s. Sulfuric acid (500 µL; Wako Pure Chemical Industries) was added to the sample and vortexed until it produced heat. After incubation at room temperature for 20 min, the OD_{490} was measured. Glucose concentration was determined using an ACCU-CHEK strip F (Roche Diagnostics K.K., Tokyo, Japan).

3. Results

3.1. *Growth of lactic acid bacteria in vegetable and fruit juices*

We previously reported three strains of lactic acid bacteria, *L. carnosum* #7-2, *L. gelidum* #4-2, and *L. mesenteroides* 8/11-3, with high innate immunity-stimulation activities. These lactic acid bacteria grow in milk. In the present study, we examined whether those bacterial strains could grow in vegetable or fruit juice. The concentration of *L. mesenteroides* 8/11-3 increased to more than 1.0×10^6 colony forming units (cfu)/mL after culture for 70 h in broccoli, mixed vegetable, and apple juices (Table 2). Moreover, the concentration of this strain increased to 1.0×10^6 cfu/mL after culture for 94 h in bitter melon and mandarin orange juices

Table 2. Growth of lactic acid bacteria in vegetable and fruit juices without pH adjustment

Strains	Juices (Before neutralization)	Bacterial concentration in juice (cfu/mL)	
		After 70 h	After 94 h
L. carnosum #7-2	Bitter melon	$< 1.0 \times 10^3$	N.D.
	Broccoli	$< 1.0 \times 10^3$	N.D.
	Mixed vegetable	$< 1.0 \times 10^3$	N.D.
	Apple	$< 1.0 \times 10^3$	N.D.
	Mandarin orange	$< 1.0 \times 10^3$	N.D.
	Kiwi fruit	$< 1.0 \times 10^3$	N.D.
L. gelidum #4-2	Bitter melon	$< 1.0 \times 10^3$	N.D.
	Broccoli	1.0×10^5	1.5×10^4
	Mixed vegetable	1.0×10^4	1.7×10^4
	Apple	$< 1.0 \times 10^3$	N.D.
	Mandarin orange	$< 1.0 \times 10^3$	N.D.
	Kiwi fruit	$< 1.0 \times 10^3$	N.D.
L. mesenteroides 8/11-3	Bitter melon	1.0×10^5	1.0×10^6
	Broccoli	1.0×10^6	1.4×10^7
	Mixed vegetable	1.0×10^6	4.6×10^7
	Apple	1.0×10^6	1.2×10^7
	Mandarin orange	1.0×10^3	1.0×10^6
	Kiwi fruit	$< 1.0 \times 10^3$	N.D.

Vegetable and fruit juices were autoclaved at 121°C for 20 min. Lactic acid bacteria were precultured anaerobically in MRS liquid media at 30°C. Aliquots (10 mL) of vegetable and fruit juices with 50 µL of the preculture was further cultured at 30°C. (N.D., not determined).

Table 3. Growth of lactic acid bacteria in vegetable and fruit juices with pH adjustment

Strains	Juices (After neutralization)	Bacterial concentration in juice (cfu/mL)	
		After 24 h	After 48 h
L. carnosum #7-2	Kiwi fruit	1.0×10^2	N.D.
	Apple ①	1.0×10^4	N.D.
	Orange ①	N.D.	1.4×10^8
	Grapefruit	N.D.	1.4×10^7
L. gelidum #4-2	Kiwi fruit	N.D.	1.4×10^5
	Apple ①	N.D.	6.7×10^6
	Orange ①	N.D.	1.1×10^8
	Grapefruit	N.D.	2.1×10^8
L. mesenteroides 8/11-3	Kiwi fruit	N.D.	4.7×10^6
	Apple ①	N.D.	2.3×10^8
	Orange ①	N.D.	1.1×10^9
	Grapefruit	N.D.	1.1×10^8

Adjustment of fruit juice pH was conducted by adding 10N NaOH solution. Neutralized juices into which preculture was added was cultured at 30°C. One hundred grams of kiwi fruit were cut, and 500 ml of water was added and crushed by a juicer and neutralized. Apple ① (Tokyo Meiraku Co Ltd), orange ①, and grapefruit juices were neutralized before use. These fruit juices were autoclaved at 121°C for 20 min. (N.D., not determined).

Table 4. Change of sugar concentration and pH in fruit juices with lactic acid bacterial culture

Strains	Juices (After neutralization)	Culture time (h)	Concentration of total sugar (mg/mL)		Concentration of glucose (mg/mL)		pH of juices	
			Without bacteria	With bacteria	Without bacteria	With bacteria	Without bacteria	With bacteria
L. carnosum #7-2	Kiwi fruit	48	15	N.D.	19	N.D.	7	N.D.
	Apple ①	48	79	N.D.	2.9	N.D.	6	N.D.
	Apple ②	72	72	67	N.D.	N.D.	7	4.5
	Orange ①	48	72	45	2.8	<0.1	6	N.D.
	Orange ②	72	69	49	N.D.	N.D.	7	4
	Orange ③	24	95	75	N.D.	N.D.	7	6
	Orange ④	48	95	65	N.D.	N.D.	7	4
	Orange ⑤ (slow juicer)	24	93	77	N.D.	N.D.	7	5
	Orange ⑥ (slow juicer)	48	93	68	N.D.	N.D.	6	4
	Grapefruit	48	65	44	2.4	N.D.	6	N.D.
L. gelidum #4-2	Kiwi fruit	48	15	8	19	1.9	7	N.D.
	Apple ①	48	79	N.D.	2.9	N.D.	6	N.D.
	Apple ②	72	72	64	N.D.	N.D.	7	4
	Orange ①	48	72	50	2.8	< 0.1	6	N.D.
	Orange ②	72	69	60	N.D.	N.D.	7	4
	Orange ③	24	95	62	N.D.	N.D.	7	4
	Orange ④	48	95	68	N.D.	N.D.	7	4
	Orange ⑤ (slow juicer)	24	93	70	N.D.	N.D.	7	5
	Orange ⑥ (slow juicer)	48	93	75	N.D.	N.D.	6	4
	Grapefruit	48	65	46	2.4	< 0.1	6	N.D.
L. mesenteroides 8/11-3	Kiwi fruit	48	15	2	19	1.4	7	N.D.
	Apple ①	48	79	59	2.9	4.3	6	N.D.
	Apple ②	72	72	62	N.D.	N.D.	7	4
	Orange ①	48	72	34	2.8	1.7	6	N.D.
	Orange ②	72	69	39	N.D.	N.D.	7	3
	Orange ③	24	95	49	N.D.	N.D.	7	3
	Orange ④	48	95	47	N.D.	N.D.	7	3
	Orange ⑤ (slow juicer)	24	93	73	N.D.	N.D.	7	3
	Orange ⑥ (slow juicer)	48	93	54	N.D.	N.D.	6	3
	Orange ⑦ (slow juicer)	48	98	55	N.D.	N.D.	6	3
	Grapefruit	48	65	46	2.4	<0.1	6	N.D.

Neutralized juices into which preculture were added was cultured at 30°C for 2-3 days. Apple juice ①, ②, orange juice ①, ②, ③, and ④ were neutralized and autoclaved before use. Orange ⑤, ⑥, and ⑦(US) were prepared by slow juicer. Oranges (⑤, ⑥: from Australia, ⑦: from US) were crushed by slow juicer, and neutralized. These juices were autoclaved before use. (N.D., not determined).

(Table 2). The concentration of *L. mesenteroides* 8/11-3 after culture for 70 h in kiwi fruit juice was less than 1.0×10^3 cfu/mL (Table 2). The concentrations of *L. carnosum* #7-2 and *L. gelidum* #4-2 after culture for 70 h in bitter melon, broccoli, mixed vegetable, apple, mandarin orange, and kiwi fruit juices were lower than 1.0×10^5 cfu/mL (Table 2).

Neutralizing tomato juice with sodium hydrogen carbonate facilitates the growth of lactic acid bacteria (*4*). Therefore, we examined whether these lactic acid bacteria could grow in neutralized fruit juices. The concentrations of all three strains were increased to 1.4 $\times 10^7$ cfu/mL after 48 h in orange and grapefruit juices (Table 3). Moreover, the concentrations of *L. gelidum* #4-2 and *L. mesenteroides* 8/11-3 in neutralized kiwi fruit and apple juices increased to 1.4×10^5 cfu/mL after 48 h (Table 3). The pH of apple and orange juices decreased after the growth of lactic acid bacteria (Table 4).

3.2. *Decreased sugar concentration after growth of lactic acid bacteria in vegetable and fruit juices*

Bacterial growth consumes sugars in culture media. We determined the total sugar and glucose concentrations in neutralized juices in which the three strains of lactic acid bacteria were cultured. The concentration of total sugar decreased under all conditions in which bacteria grew (Table 4). In particular, the total sugar content of kiwi fruit juice in which *L. mesenteroides* 8/11-3 grew decreased to 13% that of the original level. Glucose concentrations of juices in which *L. mesenteroides* 8/11-3 grew, except apple juice, decreased (Table 4). In particular, the glucose concentration decreased to below the detection limit in orange and grapefruit juices in which *L. carnosum* #7-2 and *L. gelidum* #4-2 grew, and in kiwi fruit and grapefruit juices in which *L. mesenteroides* 8/11-3 grew. These results indicate that growth of these three strains of lactic acid bacteria, *L. carnosum* #7-2, *L. gelidum* #4-2, and *L. mesenteroides* 8/11-3, decreased the concentration of total sugars and glucose in fruit juices.

4. Discussion

In this paper, we demonstrated that the growth of *L. carnosum* #7-2, *L. gelidum* #4-2, and *L. mesenteroides* 8/11-3 decreased sugar concentrations in vegetable and fruit juices. These three strains are functional lactic acid bacteria with high innate immune system-stimulating activity, as determined in silkworms by a muscle contraction assay. Vegetable and fruit juices are generally acidic, and bacteria generally do not grow well in these juices. We showed that neutralizing the juices with sodium hydroxide provides the appropriate conditions for growing the three strains of lactic acid bacteria. Besides a low pH, insufficient nutrition or the presence of bactericidal substances inhibit bacterial growth. To solve these problems, it is necessary to identify such factors in other juice samples.

Excessive intake of sucrose, glucose, and fructose from fruit juices is thought to contribute to the development of diabetes and obesity (*5-8*). We propose here that fermenting juices using lactic acid bacteria may solve this problem. The lactic acid bacterium *L. mesenteroides* 8/11-03 strain grew in bitter melon and broccoli juices. Therefore, this lactic acid bacterium may reduce sugars not only in fruit juices, but also in vegetable juices. Lactic acid bacteria decrease the sugar content of vegetable juices, fruit juices, and mixed juices (*4,9-11*). In addition, the lactic acid bacteria *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Lactobacillus plantarum* decrease the glucose and fructose concentrations of a juice made from rice flour (*12*). To our knowledge, there are no reports that "functional lactic acid bacteria" decrease the sugar content of juices. The presence of the functional lactic acid bacteria may confer additional beneficial effects to juices for maintaining human health.

The Ministry of Health, Labor, and Welfare in Japan reports that fermented milk containing a minimum of 8.0% milk solids-not-fat content should contain at least 1×10^7 cfu/mL of lactic acid bacteria (*13*). In this study, we demonstrated that the concentrations of the three strains of lactic acid bacteria increased to 1×10^7 cfu/mL in vegetable and fruit juices after the pH was neutralized. Lactic acid bacteria of the *Leuconostoc* genus have long been used for manufacturing fermented foods, indicating that humans have experience ingesting those lactic acid bacteria. Therefore, we propose here the manufacture of vegetable and fruit juices containing these lactic acid bacteria as fermented foods that may be beneficial to human health.

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