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Technical paper

Storage of a Broccoli Lactic Acid Bacteria Drink

Riichiro OHBA,[1] Masaki IIO[2] and Yoshiharu SASAKI[1]

[1]*Sojo University, 4-22-1, Ikeda, Kumamoto 860-0082, Japan*
[2]*Fine Chemical Laboratory Co., Ltd., 5-7-8, Shimosinjo, Higashiyodogawa-ku, Osaka 533-0021, Japan*

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A lactic acid bacteria drink from a vegetable (broccoli) with high nutritional value and various physiological functionalities was developed, and the preserving property of the drink was studied. When this the broccoli lactic acid bacteria drink was stored at 5°C and citric acid was added to it, the drink had a good taste and the number of lactic acid bacteria remained over 10^7 CFU/ml for about 10 days. In addition, the broccoli pasteurized lactic acid bacteria drink was developed in order to extend the storage time. The lactic acid bacteria were sufficiently killed after heating at 75°C for 40 min, when pasteurization condition of the lactic acid bacteria drink was examined. However, vitamin C content was lowered to about 60%. Then, when erythritol was added to the pasteurized lactic acid bacteria drink, the storage time was extended to 3 weeks, and the taste and aroma also improved. We found the fact that the broccoli pasteurized lactic acid bacteria drink suppressed the increase in acetaldehyde, ethanol and acetic acid which cause to the quality deteriorate in storage.

Keywords: lactic acid bacteria drink from a vegetable, storage time, citric acid, erythritol, pasteurized lactic acid bacteria drink from broccoli

Lactic acid bacteria drinks contain the nutrients such as calcium, phosphorus and protein. Many reports have been published indicating these drinks have physiological functionalities (Labell, 1989; Nakajima *et al*., 1992; Mann & Spoerry, 1974; Reddy *et al*., 1973; Shahani *et al*., 1976; Takano *et al*., 1985; Yamamoto *et al*., 1994a; 1994b). Thus, the lactic acid bacteria drink is a highly superior beverage, however, it contain few vitamins and no fiber at all. On the other hand, vegetables contain important nutrients such as vitamins, minerals, and fiber. It is apparent that vegetables have physiological functions of maintaining health and disease prevention (Bors & Saran, 1987; Cao *et al*., 1996; Kada *et al*., 1982; Wattenberg, 1985).

In our previous experiment, we developed a new compound beverage which mixed a lactic acid bacteria drink and a vegetable (broccoli). This drink contains vitamin C and more inorganic ingredients than general lactic acid bacteria drinks (Ohba & Iio, 2000). However, in the place market, shelf life is important, and this item is difficult to sell, has a short shelf life and a high cost. We therefore looked into refrigerating this broccoli lactic acid bacteria drink. We measured the aroma component and the number of lactic acid bacteria and performed a sensory evaluation. To extend the shelf life of the drink, ascorbic acid which is used to retain flavor, citric acid used to improve the texture of dairy products and sorbic acid which is used to prevent decay of dairy products was added to it. Acidification, however, degrades the flavor, and is observed even during refrigeration (Laye *et al*., 1993). To avoid this phenomenon, we also developed a pasteurized lactic acid bacteria drink, and experimented in enhancing the sugar concentration to prevent contamination after the product had been opened.

E-mail: ohba@bio.sojo-u.ac.jp

Materials and Methods

Method of producing the broccoli lactic acid bacteria drink Broccoli powder was dissolved in water and a suspended solution of 4% was prepared. The suspended solution was filtered (Toyo No. 2) and to 160 ml of the filtered solution was added 10 g of skim milk powder, 10 g of sugar and 8 g of brown sugar. The mixture was pasteurized at 70°C for 15 min, 40 ml of starter was added (*Streptococcus lactis* subsp. *cremoris* H-61) and the whole was fermented at 30°C for 36 h. Thereafter, 0.5 g of citric acid, 0.5 g of ascorbic acid and 0.01 g of sorbic acid were added.

Measurement of viable cell count and pH Plate count agar with BCP (Nissui, Tokyo) was used to enumerate viable *S. lactis* subsp. *cremoris* H-61 in the broccoli lactic acid bacteria drink. Plates inoculated by the smear culture method were incubated for 48 h at 37°C and colonies were counted with the assistance of a Colony Counter (Shibata, Tokyo). The pH meter F-21 (Horiba, Kyoto) was used to measure pH.

Analysis of volatile compounds Four milliliters of the broccoli lactic acid bacteria drink refrigerated for 0, 1 and 7 days and 1 ml of 0.2% n-amyl alcohol were mixed, sealed with a rubber cap and incubated for 30 min at 80°C. Two milliliters of headspace gas was fractionated by gas chromatography: Model: GC-14A (Shimadzu, Kyoto), Column: CBP20-S25-050, 25 mm ×0.33 mm, column temperature: 50–200°C (5°C/min), detector: FID (230°C), carrier gas: N_2 (1.35 mi/min), sensitivity: $10^0 \times 2^3$. Quantification was based on the internal standard method. The concentration of each volatile compound was calculated by comparison of retention time and peak area by the standard materials of acetaldehyde, ethanol, diacetyl and acetoin.

Sensory evaluations During the refrigerated storage, the drinks were evaluated by 10 members of the laboratory staff (5 males and 5 females; age; 22–35), taste and flavor were evaluated respectively in 4 steps.

Procedure for making the broccoli pasteurized lactic acid bacteria drink After the broccoli lactic acid drink was prepared, it was pasteurized at 60–75°C for 20–40 min and then the results of pasteurization, using viable cell count and vitamin C content were analyzed. Vitamin C content was measured according to the hydrazine coloration method (Ohba & Iio, 2000).

Examination of the addition of erythritol Erythritol was added to the prepared drink in concentrations of 0, 5, 10 and 15%. It was then sterilized and at the end of each storage period, a sensory test was performed.

HPLC analysis of organic acids The broccoli lactic acid bacteria drink was diluted with deionized water and centrifuged for 10 min at 3500 rpm. Supernatant fractions were filtered through a 0.2 μm membrane filter (Toyo, Tokyo). The filtered supernatant fractions (20 μl) were then injected into a HPLC; model: SPD-10AV (Shimadzu), column: YMC-Pack ODS-AQ-304 (300 mm×4.6 mm), solvents: 20 mM H_3PO_4-NaH_2PO_4 (pH 2.8), flow: 1.0 ml/min, temperature: 15°C, sensitivity: ATTEN; 6 AUX RANGE; 5. Oxalic acid, tartaric acid, pyruvic acid, malic acid, malonic acid, lactic acid, acetic acid, citric acid, fumaric acid and propionic acid were prepared in three concentrations and chromatographed to determine retention times. Quantification was based on the external standard method. Best fit, linear regression lines were obtained for peak height vs. concentration for each compound. These compounds were detected and quantified by monitoring the effluent at 220 nm.

Results and Discussion

Effect on preservation of the broccoli lactic acid bacteria drink by food additive

Change of viable cell count and pH The drink was preservated in refrigerated storage and every few days, viable cells were counted (Fig. 1). There were no obvious differences found in the pH of the drink samples (pH 4.0–4.1), whereas a tendency was observed for viable cell counts of the control and the ascorbic acid additive sample to decrease after 3 days. The viable cell count was approximately 10^7 CFU/ml at 7 days. A decreasing tendency was also observed in the viable cell counts of the citric acid and sorbic acid additive samples after 5 days. It is thus apparent that the extinction rate of lactic acid bacteria was inhibited by the citric acid and the sorbic acid.

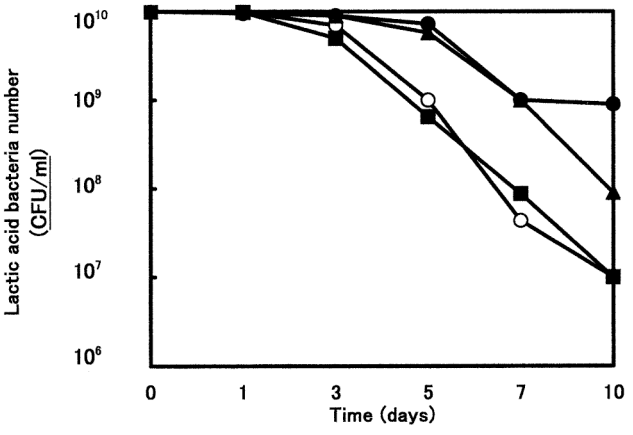

Fig. 1. Effects of food additives on the number of lactic acid bacteria in the broccoli lactic acid bacteria drink. Preservation temperature, 5°C. ○, Control; ●, Sorbic acid; ▲, Citric acid; ■, Ascorbic acid.

Table 1. Effects of food additive on characteristics of the broccoli lactic acid bacteria drink.

Food additive	No addition			0.5% Citric acid		
Storage time (days)	0	7	10	0	7	10
Acetaldehyde (ppm)	24.2	11.4	11.0	24.2	41.4	45.6
Ethanol	0.8	1.9	3.4	2.0	3.9	10.1
Diacetyl	1.5	1.7	2.0	4.5	5.9	4.3
Acetoin	3.7	1.9	1.8	3.4	3.3	2.7
Sensory test[a)]						
Aroma	+++	++	++	+++	+++	++
Taste	+++	++	+	+++	+++	+++

Food additive	0.5% Ascorbic acid			0.01% Sorbic acid		
Storage time (days)	0	7	10	0	7	10
Acetaldehyde (ppm)	24.2	41.6	51.3	24.2	19.4	18.8
Ethanol	0.8	8.9	32.7	0.8	3.9	4.2
Diacetyl	1.5	2.5	2.5	1.5	2.4	2.3
Acetoin	3.7	0.7	0.6	3.7	3.0	2.9
Sensory test[a)]						
Aroma	+++	++	+	+++	++	++
Taste	++	+	–	+++	+++	++

Food additives were added to lactic acid bacteria drink after fermentation. Storage temperature was 5°C.
Volatile components were determined by gas chromatography.
[a)]+++; very good, ++; good, +; bad, –; very bad.

Analysis of volatile compounds and sensory test The concentration of volatile compounds in all refrigerated samples changed (Table 1). For acetaldehyde, the control and the sorbic acid additive decreased, whereas the citric acid additive and the ascorbic acid additive increased. For ethanol and diacetyl, all samples increased. For acetoin, all samples decreased. These results agreed with a previous article (Laye *et al*., 1993). These volatile compounds were detected in earlier reports (Laye *et al*., 1993; Bottazzi & Dellaglio, 1967; Bottazzi & Vescovo, 1969; Ha *et al*., 1992; Hamdan *et al*., 1971; Kang *et al*., 1988; Keenan & Bills, 1968).

In the sensory test, the control and ascorbic acid additive sample deteriorated remarkably during preservation. In the sensory test at 10 days, volatile compounds were changing, but the citric acid additive was the best. This additive was therefore determined to be the best because it maintained the taste up to 10 days.

Preparation method of the broccoli pasteurized lactic acid bacteria drink

Examination of the lactic acid bacteria pasteurization condition It was shown that it was possible to preserve the broccoli lactic acid bacteria drink for 10 days when citric acid was added. However, the preservation period must be lengthened because it is difficult to sell with a short shelf life when long distance transport is required. We therefore attempted to produce a pasteurized lactic acid bacteria drink, and first examined the condition governing lactic acid bacteria pasteurization (Table 2). This condition was determined from the viable cell count of the lactic acid bacteria and the vitamin C content. Lactic acid bacteria died out after heating at 60°C for 70 min, but only a little more vitamin C (5.4 μg/ml) remained than before pasteurization (18.9 μg/ml), whereas after heating at 75°C for 40 min lactic acid bacteria died out and more vitamin C remained than heating at 60°C for 70 min (7.2 μg/ml). Heating at a temperature of 80°C

Table 2. Effects of various pasteurizing conditions on cell number and vitamin C content in the broccoli lactic acid bacteria drink after fermentation.

Pasteurizing conditions	Lactic acid bacteria number (CFU/ml)	Vitamin C (μg/ml)
Control	2.5×10^{10}	18.9 (100)
60°C, 20 min	3.9×10^{4}	12.3 (65)
60°C, 30	500	10.5 (56)
60°C, 40	255	9.3 (49)
60°C, 50	129	8.5 (45)
60°C, 60	32	6.8 (36)
60°C, 70	0	5.4 (29)
70°C, 20min	125	11.1 (59)
70°C, 30	99	9.1 (48)
70°C, 40	19	7.9 (42)
70°C, 50	0	7.9 (34)
75°C, 20min	95	10.4 (55)
75°C, 30	42	8.5 (45)
75°C, 40	0	7.2 (38)

( ): control was expressed as 100%.

seemed unsuitable because aggregation of protein in the drink was observed after heating. Therefore, more vitamin C would remain if it were pasteurized at 75°C for 40 min.

Examination of erythritol concentration We conducted an experiment which made sugar concentration high to prevent contamination after the product was opened. Maltitol, sorbitol and erythritol, which have no calories, were added to the broccoli pasteurized lactic acid bacteria drink. The result was that it was as good as the most sensory of erythritol. Table 3 shows the effect of erythritol concentration on quality of the broccoli pasteurized lactic acid bacteria drink. The pH during refrigeration did not show any change. In the sensory test, the sweetness was too strong at 15% erythritol concentration, but at 10% the sweetness was good. Taste and aroma of the drink with no erythritol was good for 14 days, whereas the drink with 10% erythritol concentration was good for 21 days.

Analysis of volatile compounds and organic acids in the broccoli lactic acid bacteria drink and the broccoli pasteurized lactic acid bacteria drink

Analysis of volatile compounds and sensory test Table 4 shows a comparison of volatile compounds in the broccoli lactic acid bacteria drink and the broccoli pasteurized lactic acid bacteria drink. In the sensory test, the broccoli lactic acid bacteria drink was good in taste and aroma for 7 days, whereas the broccoli pasteurized lactic acid bacteria drink was good for 21 days.

In the volatile compounds, acetaldehyde and ethanol of the broccoli lactic acid bacteria increased with the preservation time, while the broccoli pasteurized lactic acid bacteria drink did not show this tendency.

Analysis of organic acids Table 5 shows a comparison of organic acids in the two drinks. After fermentation, lactic acid increased about 3 fold. The increase in the citric acid was be-

Table 3. Effects of erythritol on characteristics of the broccoli pasteurized lactic acid bacteria drink.

Erythritol (%)	Storage time (days)	Sensory test[a)]	
		Aroma	Taste
0	0	++	++
	7	++	++
	14	++	+
	21	+	–
5	0	+++	+++
	7	++	+++
	14	++	++
	21	+	+
10	0	+++	+++
	7	+++	+++
	14	++	+++
	21	++	++
15	0	++	+
	7	++	+
	14	++	+
	21	+	+

[a)]+++; very good, ++; good, +; bad, –; very bad.

Table 4. Changes of volatile components in the lactic acid bacteria drink and the broccoli pasteurized lactic acid bacteria drink during preservation.

A)

Storage time (days)	0	7	14	21
Acetaldehyde (ppm)	22.2	40.3	58.3	61.2
Ethanol	1.1	2.8	10.0	12.9
Diacetyl	3.6	4.6	4.4	4.0
Acetoin	3.2	3.4	2.6	2.0
Sensory test[a)]				
Aroma	+++	++	+	+
Taste	+++	+++	+	–

B)

Storage time (days)	0	7	14	21
Acetaldehyde (ppm)	20.1	22.3	21.5	20.6
Ethanol	0.9	1.2	2.5	3.3
Diacetyl	3.4	3.8	3.3	2.8
Acetoin	3.2	2.0	1.8	1.5
Sensory test[a)]				
Aroma	+++	+++	++	++
Taste	+++	+++	+++	++

Storage temperature was performed at 5°C. Volatile components were determined by gas chromatography. A) The broccoli lactic acid bacteria drink. B) The broccoli pasteurized lactic acid bacteria drink. [a)]+++; very good, ++; good, +; bad, –; very bad.

Table 5. Changes of organic acid in the broccoli lactic acid bacteria drink and the broccoli pasteurized lactic acid bacteria drink during preservation.

A)

Storage time (day)	0	7	14	21
Oxalic acid (mg/ml)	0.06	0.05	0.07	0.06
Tartaric acid	0.75	0.91	1.17	1.07
Pyruvic acid	0.15	0.13	0.18	0.16
Malic acid	0.47	0.41	0.58	0.51
Malonic acid	0.15	0.16	0.17	0.15
Lactic acid	13.24	13.44	14.17	15.56
Acetic acid	0.80	0.90	0.94	1.79
Citric acid	4.14	4.31	5.02	4.83
Fumaric acid	0.02	0.02	0.02	0.02
Propionic acid	2.48	2.71	2.67	2.48

B)

Storage time (day)	0	7	14	21
Oxalic acid (mg/ml)	0.06	0.06	0.05	0.06
Tartaric acid	0.93	1.23	1.24	1.40
Pyruvic acid	0.07	0.09	0.09	0.10
Malic acid	0.70	0.49	0.49	0.35
Malonic acid	0.15	0.15	0.17	0.19
Lactic acid	12.92	13.60	13.35	14.25
Acetic acid	0.69	0.71	0.65	0.70
Citric acid	4.37	4.34	4.81	5.45
Fumaric acid	0.02	0.02	0.02	0.02
Propionic acid	2.96	2.44	2.47	2.68

Storage temperature was performed at 5°C. Organic acid contents were determined by HPLC. A) The broccoli lactic acid bacteria drink. B) The broccoli pasteurized lactic acid bacteria drink.

cause citric acid was added after the fermentation. Acetic acid concentration of the broccoli lactic acid bacteria drink increased with preservation time. The increase of acetic acid in preservation time from 14 to 21 days seems to have had an effect on the taste and aroma of this drink. On the other hand, acetic acid concentration during preservation time in the broccoli pasteurized lactic acid bacteria drink did not show the same increase tendency. No obvious change in any other organic acid was seen.

In conclusion, it seems to be important that the increase during preservation time of acetaldehyde, ethanol and acetic acid were inhibited. We repressed production of these compounds by pasteurizing lactic acid bacteria, because those compounds in small amounts are generated at the refrigeration temperature by living lactic acid bacteria. We were therefore able to create a drink which maintained its flavor for 3 weeks.

References

Bors, W. and Saran, M. (1987). Radical scavenging by flavonoid antioxidants. *Free Radical Res. Commun*., **2**, 289–294.

Bottazzi, V. and Dellaglio, F. (1967). Acetaldehyde and diacetyl production by *S. thermophilus* and other lactic streptococci. *J. Dairy Res*., **34**, 109–114.

Bottazzi, V. and Vescovo, M. (1969). Carbonyl compounds produced by yogurt bacteria. *Neth. Milk Dairy J*., **23**, 71–78.

Cao, G., Sofic, E. and Prior, R.L. (1996). Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem*., **44**, 3426–3431.

Ha, E.Y.W., Morr, C.V. and Seo, A. (1992). Isoflavone aglucones and volatile organic compounds in soybeans: effects of soaking treatments. *J. Food Sci*., **57**, 414–417.

Hamdan, I.Y., Kunsman Jr., J.E. and Deane, D.D. (1971). Acetaldehyde production by combined yogurt cultures. *J. Dairy Sci*., **54**, 1080–1082.

Kada, T., Inoue, T. and Namiki, M. (1982). Environmental desmutagens and antimutagens. In "Environmental Mutagenesis and Plant Biology," New York, pp. 133–152.

Kang, Y.J., Frank, J.F. and Lillard, D.A. (1988). Gas chromatographic detection of yogurt flavor compounds and changes during refrigerated storage. *Cult. Dairy Prod. J*., **23**, 6–10.

Keenan, T.W. and Bills, D.D. (1968). Metabolism of volatile compounds by lactic starter culture microorganisms. a review. *J. Dairy Sci*., **51**, 1561–1567.

Labell, F. (1989). Yogurt cultures offer health benefits—Biotechnology to transform the yogurt of the future. *Food Process*., **50**, 130–138.

Laye, I., Karleskind, D. and Morr, C.V. (1993). Chemical, microbiological and sensory properties of plain nonfat yogurt. *J. Food Sci*., **58**, 991–995.

Mann, G.V. and Spoerry, A. (1974). Studies of a surfactant and cholestereaemia in the Massai. *Am. J. Clin. Nutr*., **27**, 264–269.

Nakajima, H., Suzuki, Y., Kaizu, H. and Hirota, T. (1992). Cholesterol lowering activity of ropy fermented milk. *J. Food Sci*., **57**, 1327–1329.

Ohba, R. and Iio, M. (2000). Optimum conditions for making lactic acid beverage using broccoli powder. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **47**, 384–389 (in Japanese).

Reddy, G.V., Shahani, K.M. and Banerjee, M.R. (1973). Inhibitory effect of yoghurt on Ehrlich ascites tumor cell proliferation. *J. Natl. Cancer Inst*., **50**, 815–817.

Shahani, K.M., Vakl, J.R. and Ilara, A. (1976). Natural antibiotic activity of *Lactobacillus bulgaricus*. *Cult. Dairy Prod. J*., **11**, 14–17.

Takano, T., Arai, K., Murota, I., Hayakawa, K., Mizutani, T. and Mitsuoka, T. (1985). Effects of feeding sour milk on longevity and tumorigenesis in mice and rats. *Bifidobacteria Microflora*, **4**, 31–37.

Wattenberg, L. (1985). Chemoprevention of cancer. *Cancer Res*., **45**, 1–8.

Yamamoto, N., Akino, A. and Takano, T. (1994a). Antihypertensive effect of the peptides derived from casein by an extracellular proteinase from *Lactobacillus helveticus* CP790. *J. Dairy Sci*., **77**, 917–922.

Yamamoto, N., Akino, A. and Takano, T. (1994b). Antihypertensive effects of different kinds of fermented milk in spontaneously hypertensive rats. *Biosci. Biotechnol. Biochem*., **58**, 776–778.