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Selection of Lactic Acid Bacteria Suitable for Fermented Tomato Juice and Changes in Components Due to Fermentation

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In order to optimize production of fermented tomato juice, suitable lactic acid bacteria were selected, and the changes in constituents during fermentation were investigated. Fermentation and sensory evaluation showed that *Lactobacillus bulgaricus* IAM-1120 and *L. helveticus* JCM-1120 produced a preferable juice among ten strains of lactic acid bacteria. These two strains produced much lower diacetyl and acetoin than the other strains in tomato juice that contains a high level of citric acid. These results suggested that the minimal production of these compounds from citric acid as well as sugar was an important factor for selection of bacteria in preferable fermented vegetable juice. Moreover, a decrease in hexanal, a representative aldehyde in tomato juice, appeared to contribute to taste improvement after fermentation. The nutritional requirements for amino acids, vitamins and bases for the growth of the two bacterial strains were also characterized.

Keywords: tomato juice, lactic acid fermentation, diacetyl, acetoin, nutritional requirement, carotenoid

Recent changes in dietary patterns may be implicated as important factors in the increasing incidence of adult diseases, such as cancer and arteriosclerosis. Possible prevention through diet modification is regarded as important. Considerable attention is directed towards green and yellow vegetables, such as those with a high carotene level, for which the effectiveness as antioxidant and anti-carcinogenic agents has been confirmed (Khachik *et al.*, 1992).

Recently, antioxidant and anti-cancer effects of lycopene, a red pigment in tomato, have been noted (Sakamoto & Oshima, 1995). Previously it was shown that continuous intake of tomato juice was effective in the accumulation of lycopene and β-carotene in serum and in low-density lipoproteins (Sakamoto *et al.*, 1994; Oshima *et al.*, 1996).

We have been attempting to develop an organoleptically and physiologically improved green-yellow vegetable juice by utilizing lactic acid fermentation. In our previous report, the lactic acid fermentation of carrot juice was studied, and the lactic acid bacteria suitable for fermentation as well as the changes in juice components were evaluated (Sakamoto *et al.*, 1996).

Research on lactic acid fermentation of tomato products has not been frequently reported; most works have been focused on the deterioration caused by lactic acid bacteria during production or storage (Juven & Weisslowicz, 1981). Diacetyl and acetoin, which are formed during the growth of lactic acid bacteria, are noted as spoilage indicators. These carbonyl compounds, including acetaldehyde, are important flavor components in dairy products, such as cheese and yogurt (Kaneko *et al.*, 1990), but are regarded as undesirable factors in wine and rice wine brewing (Shimazu *et al.*, 1985). We also reported that diacetyl was the main off-flavor compound in lactic acid fermented carrot juice (Sakamoto *et al.*, 1996). In tomato, citric acid is the main organic acid and seems to serve as a substrate in the formation of diacetyl and acetoin in some lactic acid fermentation (Radler & Brauhl 1984; Kaneko *et al.*, 1990).

In order to obtain an organoleptically acceptable fermented tomato juice, suitable lactic acid bacteria were selected in this study, and the changes in the tomato juice components due to fermentation were evaluated.

Materials and Methods

Preparation of tomato juice Tomato juice was prepared by the following process using a pilot plant at Kagome Research Institute: Tomatoes (variety: Kagome-77) obtained from an experimental field were washed, crushed into small pieces (average size 8–10 mm) in a crusher, heated to 75°C by a tube-type heat exchanger and squeezed by a screwpress (Kagome Co., Ltd., Tokyo). The squeezed juice was sterilized at 110°C for 60 s and cooled with a plate-type heat exchanger to 20°C. The juice was concentrated to 17% Brix by a reverse osmosis concentrator (Kagome Co., Ltd.) with PCI membrane AFC 99 (PCI Membrane Systems Ltd., Hampshire, UK). After pasteurization at 90°C for 60 s by a surface scraped heat exchanger (Iwai Kikai-Kogyo Co., Ltd., Tokyo), the mass obtained was packed in polyethylene film bags and stored at −20°C.

Before each experiment, concentrated tomato juice was thawed in a cold room (5°C) and diluted with distilled water to give a Brix of 5.0%, being comparable to that of raw tomato juice. Diluted tomato juice was adjusted to pH 6.0 with 0.1 N $NaHCO_3$ and transferred to Erlenmeyer flasks. After pasteurization in hot water (95°C, 5 min), these samples were rapidly cooled under running tap water.

Microorganisms and media Lactic acid bacteria used were *Lactobacillus acidophilus* IFO-13951, *L. delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) IAM-1120, *L. casei* IAM-1045, *L. delbrueckii* subsp. *delbrueckii* (*L. delbrueckii*) IFO-1085, *L. helveticus* JCM-1120, *L. plantarum* IFO-3070, *Leuconostoc mesenteroides* IFO-12060, *Leu. oenos* JCM-6125, *Enterococcus faecalis* IFO-12694, and *Streptococcus thermophilus* IFO-3535.

The medium and growth conditions for lactic acid bacteria and the determination method of the viable cell population as colony forming units (cfu) were the same as in the previous paper (Sakamoto *et al*., 1996).

Pre-incubation of microorganisms and fermentation of tomato juice The conditions of pre-incubation in MRS medium (Man *et al*., 1960) were the same as those described in the previous paper (Sakamoto *et al*., 1996). Precultures were prepared by inoculation of the MRS culture into 100 ml of reconstituted tomato juice in a 200 ml Erlenmeyer flask at a ratio of 3% (v/v) and by incubation at 37°C for 8 h.

For the fermentation test, precultures were inoculated at a ratio of 3% (v/v) into 200 ml of prepared tomato juice in a 500 ml Erlenmeyer flask. After fermentation at 37°C for 20 h in an incubator, all samples, including the non-fermented control without pH adjustment, were transferred to 200 ml glass bottles and pasteurized at 95°C for 5 min. They were stored in a cold room (5°C) until analysis.

Chemical analysis Total acidity, pH, organic acids (citric, acetic, lactic and malic), carbohydrates (glucose and fructose) and amino acid composition were determined by the same methods as previously reported (Sakamoto *et al*., 1996).

Volatile analysis Analyses of volatile components were performed by a GC-MS method. Tomato juice was centrifuged at 8,000 rpm for 5 min, and the supernatant was diluted at proportions of 0.7 : 10 with distilled water. A mixture of 10 ml of diluted sample and 5 μl of *o*-dichlorobenzene/benzyl alcohol (0.012%, v/v) as the internal standard was placed into a 25 ml glass tube. The conditions of purging and trapping onto a Tenax tube and the method of GC-MS analysis were the same as in the previous paper (Sakamoto *et al*., 1996). Determination of diacetyl and acetoin concentrations was done based on standard curves obtained using 0.1, 0.5, 1.0, 2.0 ppm diacetyl solutions and 1.0, 5.0, 10.0, 20.0 ppm acetoin solutions.

Sensory evaluation The sensory evaluation was done by the paired-preference test using non-fermented and pH-unadjusted tomato juice (control) by 16 trained sensory panelists. Samples were kept in glass bottles at 20°C and were tested under a red lamp to disguise color differences. Statistical analyses were carried out by two-paired test.

Color and carotenoid analysis The color of tomato juice was measured by a Hunter color difference meter ND-Σ80 (Nippon Denshoku Ind. Co., Ltd., Tokyo) using red standard panel as reference. The content of lycopene and β-carotene and the composition of carotenoids were analyzed by reversed phase HPLC as reported in our previous paper (Sakamoto *et al*., 1996). Calibration curves of lycopene and β-carotene based on peak area were established using lycopene prepared from tomato paste and β-carotene (Sigma Chemical Co., St. Louis, MO, USA), which were adjusted to 1.0, 2.0, 5.0, 10.0 mg% and 0.1, 0.5, 1.0 mg% solution in *n*-hexane.

Nutritional requirement The requirements for amino acids, vitamins and bases were examined in accordance with the experimental manual for lactic acid bacteria (Kosaki *et al*., 1992).

Results and Discussion

Selection of lactic acid bacteria suitable for fermented tomato juice To assess the suitability of various strains for fermentation of tomato juice, the values of acidity, pH and viable cell number after 20 h of fermentation were compared (Table 1). The strains examined grew well in the juices, except for *L. acidophilus* IFO-13951. For sensory evaluation, diacetyl and acetoin contents and the organic acid composition of the fermented juice were determined (Table 2). Tomato juice samples fermented by *L. bulgaricus* IAM-1120 and *L. helveticus* JCM-1120, which contained less amounts of diacetyl and acetoin compared with those fermented by the other strains, were preferred by most panelists. These strains were also preferred for the fermentation of carrot juice (Sakamoto *et al*., 1996). In contrast, *S. thermophilus* IFO-3535, which was preferred for carrot juice fermentation,

Table 1. Comparison of acidity, pH and viable cell number of tomato juice obtained by fermentation with ten different lactic acid bacteria.

Microorganism	Acidity (%)	pH	Cell number (cfu/ml)
L. acidophilus	0.57	5.10	9.8×10^7
L. bulgaricus	0.69	4.11	2.6×10^9
L. casei	0.65	4.63	6.1×10^8
L. delbrueckii	0.86	3.90	8.7×10^9
L. helveticus	0.84	4.03	3.5×10^9
L. plantarum	0.87	3.88	7.1×10^9
E. faecalis	0.81	4.21	2.7×10^9
Leu. mesenteroides	0.82	4.26	1.4×10^9
Leu. oenos	0.76	4.30	1.1×10^9
S. thermophilus	0.76	4.50	7.6×10^8

Initial cell number: $0.5\text{-}3.0\times10^6$ (cfu/ml).

Table 2. Comparison of contents of diacetyl, acetoin and organic acids and sensory evaluation of tomato juice obtained by fermentation with ten different lactic acid bacteria.

Microorganism	Diacetyl (μg/ml)	Acetoin (μg/ml)	Citric acid (%)	Lactic acid (%)	Acetic acid (%)	Sensory test[a]
L. bulgaricus	0.17	ND	0.46	0.49	ND	13*
L. casei	0.80	ND	0.43	0.58	ND	0**
L. delbrueckii	1.26	ND	0.39	0.67	ND	0**
L. helveticus	0.16	ND	0.47	0.49	ND	12*
L. plantarum	0.19	ND	0.43	0.54	ND	8
E. faecalis	0.78	ND	0.43	0.63	ND	1**
Leu. mesenteroides	0.11	ND	0.47	0.48	0.21	7
Leu. oenos	0.12	ND	0.43	0.44	0.14	6
S. thermophilus	0.32	13.40	0.46	0.45	ND	4*
control	0.12	ND	0.46	ND	ND	—

ND, not detected.

[a] Number of panelists who preferred fermented tomato juice to non-fermented one (control).

* Significant difference ($p<0.05$).

** Significant difference ($p<0.01$).

produced off-flavor in the fermented tomato juice. Because only *S. thermophilus*, among the strains tested, formed a significant amount of acetoin, acetoin could be a cause for the poor sensory evaluation. Tomato juices fermented by *L. plantarum* IFO-3070, *Leu. mesenteroides* IFO-12060 and *Leu. oenos* JCM-6125 produced an unusual flavor which could not be explained by diacetyl and acetoin. The other strains tested, which produced a strong off-flavor due to diacetyl, were unsuitable for tomato juice fermentation.

The production of diacetyl and acetoin by lactic acid fermentation is known in the substrates that contain citric acid, such as milk with citrate added (Hegazi & Abo-Elnaga, 1980; Kaneko *et al.*, 1990). Also, the decomposition of citric acid with the production of acetic acid, diacetyl and acetoin

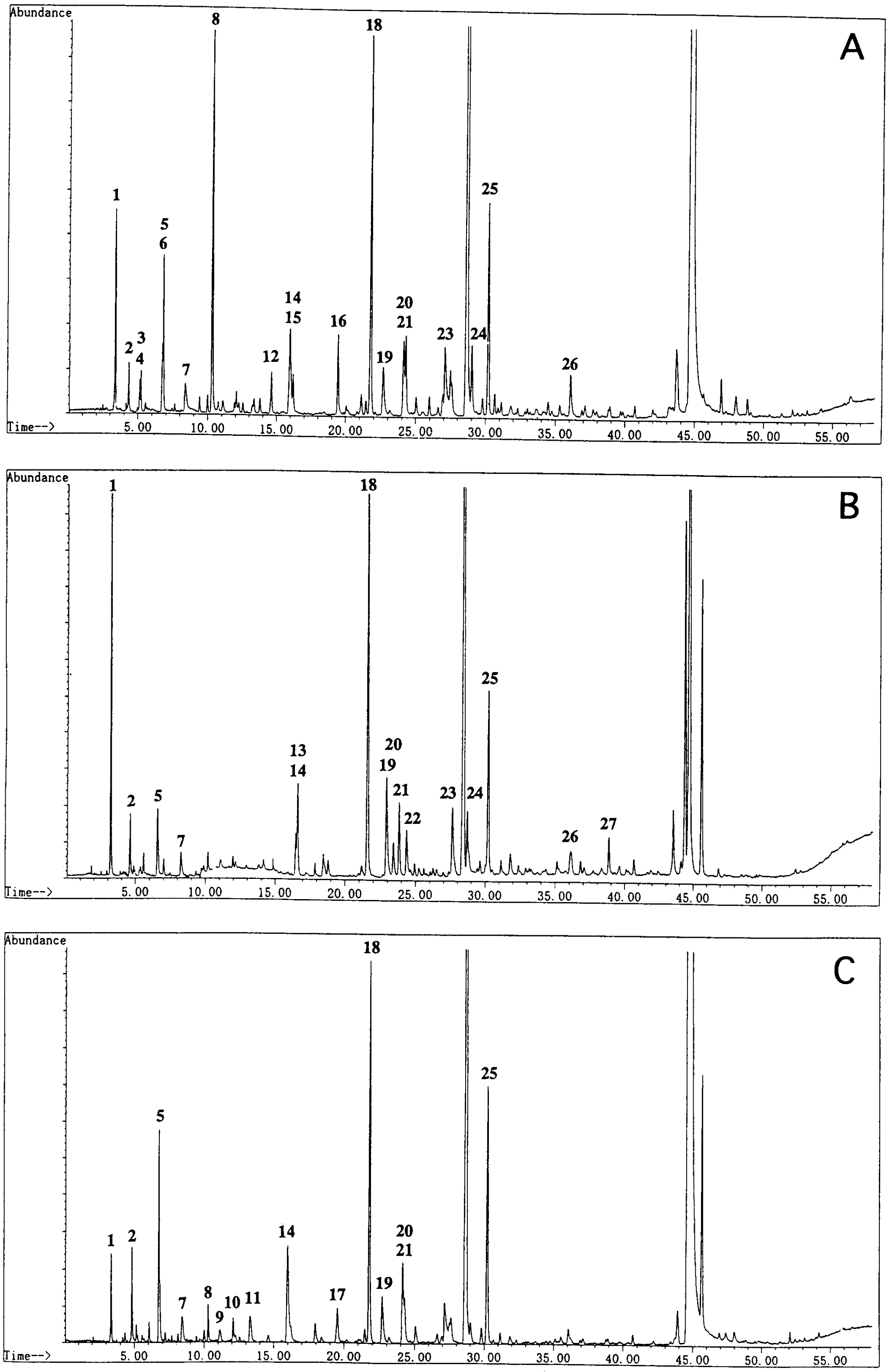

Fig. 1. Gas chromatograms of volatile compounds from non-fermented tomato juice (A), fermented with *L. bulgaricus* (B) and with *S. thermophilus* (C).

by the malo-lactic fermentation bacteria in wine production has been reported (Shimazu *et al.*, 1985). Citric acid is the major organic acid of tomato juice. The citric acid of tomato juice was consumed by *L. casei* IAM-1045, *L. delbrueckii* IFO-1085, *L. plantarum* IFO-3070 and *E. faecalis* IFO-12694 other than *Leu. oenos* JCM-6125 which was one strain in species *Leu. oenos* known as a citrate-utilizing bacteria (Radler & Brauhl 1984) (Table 2). *L. casei*, *L. delbrueckii* and *E. faecalis* produced more diacetyl in the fermented tomato juice, but *L. plantarum* and *Leu. oenos* produced minimal amount of diacetyl and acetoin. On the other hand, diacetyl and acetoin were produced without a decrease in citric acid by *S. thermophilus* IFO-3535. Although *L. plantarum*, *Leu. mesenteroides* and *Leu. oenos* are known as malo-lactic acid fermentation bacteria, a significant amount of diacetyl was not observed in fermented tomato juice. The production of acetic acid by these strains was considered to be due to hetero lactic acid fermentation.

Because *L. bulgaricus* IAM-1120 and *L. helveticus* JCM-1120 produced very low levels of diacetyl and acetoin in the fermented juice of tomato and carrot, these strains were considered to be suitable for the production of vegetable juice from various sources. However, because many factors such as pyruvate, amino acids and enzymes are involved in the production of diacetyl and acetoin (Morita, 1996), further studies are required on the production mechanism of the unfavorable components by these strains.

Specification of flavor components that cause organoleptic differences in fermented tomato juice As the fermented tomato juices produced by *L. bulgaricus* IAM-1120 and *L. helveticus* JCM-1120 were preferred by the sensory test, the changes in flavor components other than diacetyl and acetoin were analyzed by GC-MS. The gas chromatograms of non-fermented tomato juice and fermented juice with *L. bulgaricus* IAM-1120 and with *S. thermophilus* IFO-3535 are shown in Fig. 1, and the peaks determined by GC-MS analysis are summarized in Table 3. Many aldehydes, such as pentanal, hexanal, heptanal, 2*E*-hexenal, octanal, decanal, which were commonly known as tomato flavor components (Buttery *et al.*, 1990), remarkably decreased. These changes were observed with many other strains. In the juice fermented by *L. bulgaricus* IAM-1120, the disappearance of hexanal and the increase in the amount of 2-pentanone and hexanol were characteristic. Similar changes were also observed with the fermented tomato juice by *L. helveticus* JCM-1120, except for the change in the amount of 2-pentanone. Although hexanal is well known as representative green tomato flavor, the decomposition and change to hexanol by alcohol dehydrogenase of the lactic acid bacteria may contribute to the improvement of juice quality. Ketones previously found in carrot juice (Sakamoto *et al.*, 1996) were not found in the fermented tomato juice. The identification of the factors that cause the differences remain to be solved.

Table 3. Odor constituents of non-fermented and fermented tomato juice.

Peak number	Component	Relative peak area (%)[a]		
		a	b	c
1	2-Pentanone	8.66	26.37	3.74
2	2-Butanone	3.50	3.75	3.74
3	2-Methyl-1-butanal	1.14	—	—
4	3-Methyl-1-butanal	1.63	—	—
5	Diacetyl	2.64	5.13	9.01
6	Pentanal	2.81	—	—
7	2-Butanol	7.23	2.26	2.51
8	Hexanal	23.13	—	1.65
9	2-Methyl-1-propanol	—	—	1.23
10	2-Methyl-2-propanal	—	—	1.02
11	1-Butanol	—	—	0.19
12	Heptanal	2.81	—	—
13	2-Methyl-1-butanol	—	2.82	—
14	3-Methyl-1-butanol	8.48	7.05	8.10
15	2*E*-Hexenal	2.61	—	—
16	Octanal	5.13	—	—
17	Acetoin	—	—	3.18
18	6-Methyl-5-heptene-2-one	24.09	32.40	24.09
19	Hexanol	4.99	10.40	4.64
20	3*Z*-Hexenol	6.10	3.23	6.75
21	Nonanal	6.04	6.21	3.05
22	2*E*-Hexenol	—	4.11	—
23	2-Furancarboxaldehyde	7.05	7.88	—
24	Decanal	5.77	6.31	—
25	Benzaldehyde	15.17	15.46	18.27
26	2-Furanmethanol	3.78	1.10	—

a: Non-fermented tomato juice. b: Fermented by *L. bulgaricus* IAM-1120. c: Fermented by *S. thermophilus* IFO-3535.

[a] The relative peak area to that of the internal standard (I.S. peak area=100).

Quantitative characteristics of tomato juice by lactic acid fermentation The comparison of qualitative analyses of tomato juices without fermentation and fermented by *L. bulgaricus* IAM-1120 is shown in Tables 4 and 5. Of the main sugars of tomato juice, glucose and fructose, only the glucose content decreased by 51% after the fermentation. Although most strains in the species *L. bulgaricus* ferment fructose (Kandler & Weuss, 1986), it was found that *L. bulgaricus* IAM-1120 did not utilize fructose. No significant color difference between these samples was observed. The amounts of both lycopene, the major carotenoid in tomato, and β-carotene did not significantly decrease. In terms of the amino acid contents of the juice, only a decrease in alanine content was observed.

Nutritional requirement of selected lactic acid bacteria for vegetable juice *L. bulgaricus* IAM-1120 and *L. helveticus* JCM-1120 were selected as the suitable strains for lactic acid fermentation of both carrot (Sakamoto *et al.*,

Table 4. Chemical composition and color parameters in non-fermented and fermented[a] tomato juice.

	Non-fermentation	Fermentation
pH	4.15	4.07
Refractive index (%)	5.20	4.70
Acidity (%)	0.51	0.69
Glucose (%)	1.43	0.70
Fructose (%)	1.65	1.63
Citric acid (%)	0.46	0.46
Lactic acid (%)	0.00	0.49
L Value	23.94	24.00
a Value	18.68	17.70
b Value	12.53	12.42
a/*b* Value	1.49	1.43
β-Carotene (mg%)	0.66	0.62
Lycopene (mg%)	8.58	8.48

[a] Fermented with *L. bulgaricus* IAM-1120.

Table 5. Amino acid composition of non-fermented and fermented[a] tomato juice.

Compound	Non-fermentation (mg%)	Fermentation (mg%)
Alanine	7.7	1.7
Arginine	4.5	3.3
Asparagine	73.5	70.1
Aspartic acid	37.5	35.6
Cysteine	ND	ND
Cystine	ND	ND
Glutamic acid	194.2	191.2
Glycine	1.4	2.4
Histidine	0.0	0.0
Isoleucine	2.7	1.9
Leucine	2.6	2.7
Lysine	3.3	1.0
Methionine	0.0	0.0
Phenylalanine	8.5	7.6
Proline	0.9	2.9
Serine	6.9	6.2
Threonine	8.5	6.1
Tryptophan	ND	ND
Tyrosine	2.3	2.2
Valine	2.0	0.0
Total	356.5	334.8

ND, not detected.

[a] Fermented with *L. bulgaricus* IAM-1120.

Table 6. Requirements of amino acids, vitamins and bases for the growth of *L. bulgaricus* IAM-1120 and *L. helveticus* JCM-1120.

Amino acid	①	②	Vitamin	①	②
Alanine	+	−	Thiamine	−	−
Arginine	+	+	Riboflavin	+	+
Aspartic acid	+	+	Niacin	−	−
Cystine	+	+	Pantothenic acid	+	+
Glutamic acid	+	+	Pyridoxine	−	−
Glycine	−	−	Pyridoxal	−	−
Histidine	+	+	*p*-Aminobenzoic acid	−	−
Isoleucine	+	+	Folic acid	−	−
Leucine	+	+	Biotin	+	−
Lysine	−	+	**Base**	**①**	**②**
Methionine	+	−	Adenine	−	+
Phenylalanine	+	+	Guanine	−	+
Proline	−	−	Uracil	−	+
Serine	+	−	Xanthine	−	−
Threonine	+	+			
Tryptophan	+	+			
Tyrosine	+	+			
Valine	+	−			

①: *L. bulgaricus* IAM-1120. ②: *L. helveticus* JCM-1120.
+, essential; −, non-essential.

1996) and tomato juices. Although the nutritional requirement of amino acids of these species has been reported (Ledesma *et al.*, 1977), those of vitamins and bases have not yet been much studied. Therefore, the requirements for amino acids, vitamins and bases in these selected bacteria were examined using glucose as a carbon source (Table 6). *L. bulgaricus* IAM-1120 did not require glycine, lysine and proline, but required the other 15 amino acids tested. The strain did not require four bases, but required riboflavin, pantothenic acid and biotin for growth. *L. helveticus* JCM-1120 required 12 amino acids except alanine, glycine, methionine, proline, serine and valine, it required pantothenic acid and riboflavin in the vitamins, and uracil, guanine and adenine in the bases.

It was reported that most of the lactic acid bacteria require glutamic acid and valine (Morishita, 1996). However, we found that *L. helveticus* JCM-1120 did not require valine. Similarly, the lactic acid bacteria are reported to require niacin and pantothenic acid (Morishita, 1996). However, neither of the strains examined in the present study required niacin. We found that *L. helveticus* JCM-1120 required adenine, guanine and uracil, and *L. bulgaricus* IAM-1120 did not require the bases.

Conclusions

For the development of an acceptable lactic acid fermented tomato juice, the products using the strains *L. helveticus* JCM-1120 and *L. delbrueckii* subsp. *bulgaricus* IAM-1120 were selected by sensory panelists. Though tomato juice contained citric acid as the major organic acid, the selected bacterial strains produced only a small amount of diacetyl and acetoin in the fermented juice. It was, therefore, considered that the minimal production of these carbonyl compounds is the most important factor in the selection of the lactic acid bacteria. A decrease in aldehydes represented by hexanal and an increase in hexanol in fermented tomato juice produced by these strains were observed, and the changes were suggested to contribute to the taste improvement. The strains *L. helveticus* JCM-1120 and *L. bulgaricus* IAM-1120 were found to be suitable for the lactic acid fermentation of both tomato and carrot juice. The nutritional requirements for the amino acids, the vitamins and the bases of these strains were determined. Characteristically, *L. bulgaricus* IAM-1120 did not require niacin and four bases, and *L. helveticus* JCM-1120 did not require valine and niacin.

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