Fermentation for Liquid-type Yogurt with *Lactobacillus casei* 911LC

I. H. Ko, M. K. Wang, B. J. Jeon and H. S. Kwak*

Department of Food Science and Technology, Sejong University, 98 Kunja-dong, Kwangjin-ku, Seoul 143-747, Korea

ABSTRACT : This study was carried out to find the attributes for liquid-type yogurt with *Lactobacillus casei* 911LC during 72 h fermentation at 37°C. The pH decreased up to 32 h and plauteaued thereafter, and the titratable acidity increased up to 40 h. The growth of lactic acid bacteria sharply increased with 3.5×10^7 cfu/ml up to 40 h of fermentation and slowly decreased thereafter. The free amino acids produced during fermentation reached the maximum value at 44 h and gradually decreased thereafter. Bitterness sensory scores were the highest at 44 h of fermentation. In the result of electrophoresis, the band mostly disappeared at 72 h fermentation. The present data showed that the range of optimum fermentation time for liquid-type yogurt using *Lactobacillus casei* 911LC was from 40 to 44 h. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 1 : 102-106)***

Key Words : Fermentation Time, Liquid-type Yogurt, *Lactobacillus casei*

INTRODUCTION

Liquid-type yogurt had introduced in 8th century as named "Subuk yogurt" in Turkey (Rasic and Kurmann, 1978). Since the mid 1970s, liquid-type yogurt has gained widespread consumer acceptance in for Eastern contries, which was introduced by Japan. The consumption of liquid-type yogurt has increased significantly in Korea in recent years. From these data, it is evident that liquid-type yogurt is considered to play an important role in health of the people.

Liquid-type yogurt is defined as follows: 3% of solids is contained and viable counts of lactic acid bacteria should be over 10^7 cfu/ml. The quality of the liquid-type yogurt could be various by viable counts, texture and flavor, protein precipitation (Takamizawa et al., 1966), the amount of solids (Nakanishi and Yanaji, 1966), and kinds of starter and etc. (Lee et al., 1994).

Acidification of milk by fermentation is one of the oldest methods of preserving milk and imparting to it special favorable organoleptic qualities. There are many different methods of carrying out this fermentation in various parts of the world and these give rise to a range of fermented milk products, including kumiss, kefir, acidophilus milk, and different kinds of yogurts. These products vary considerably in composition, flavor, and texture, according to the nature of fermenting organisms, the type of milk and the manufacturing process used (Tamine and Deeth, 1980).

The change pattern of proteolytic activity during fermentation is of basic important for the degree of lactic acid bacteria. Proteinase activity was detected in several strains of lactobacilli and streptococci (Ezzat et al., 1985; Zourari et al., 1992). When lactic acid bacteria as a single starter culture is used for liquid yogurt making, every yogurt plant requires different lactic acid bacteria due to their yogurt quality, fermenting conditions and consumers preference etc. Therefore, characteristics of lactic acid bacteria for the yogurt is very important to figure out.

Even though the observation of different kinds of single starter culture for liquid-type yogurt was generally applied, little information is available about the yogurt made by *Lactobacillus casei*. Therefore, the objective of this study was to examine the optimum fermentation time of yogurt made by *Lactobacillus casei* 911LC.

* Corresponding Author: H. S. Kwak. Tel: +82-2-3408-3226, Fax: +82-2-497-8931, E-mail: kwakhs@sejong.ac.kr
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MATERIALS AND METHODS

Starter culture preparation

Yogurt starter culture strain, *Lactobacillus casei* 911LC, used in this study were obtained as lyophilized pure cultures from Culture Systems Inc. (Mishawaka, IN, USA). The lactic starter culture was inoculated and propagated three times in 10% (w/v) sterile reconstituted skim milk at 37°C.

The *Lactobacillus casei* 911LC was inoculated at 2.0% (v/v) into reconstituted NDM containing 16.5% skim milk, 4% glucose, and incubated at 37°C for 72 h. During the fermentation, samples were taken at 0, 12, 32, 36, 40, 44, 48, 60 and 72 h.

Microbiological analyses

Lactic acid viable count was determined by BCP agar (Eiken Co., Tokyo, Japan). One gram of yogurt samples stored for each time of the fermentation as mentioned above were diluted with 9 ml of sterile peptone and water diluent. Subsequent serial dilutions of each sample were plated in triplicate and incubated at 37°C for 72 h.

Chemical analyses

pH values of the yogurt samples were measured using a pH meter (Orion model 900A, USA). The titratable acidity

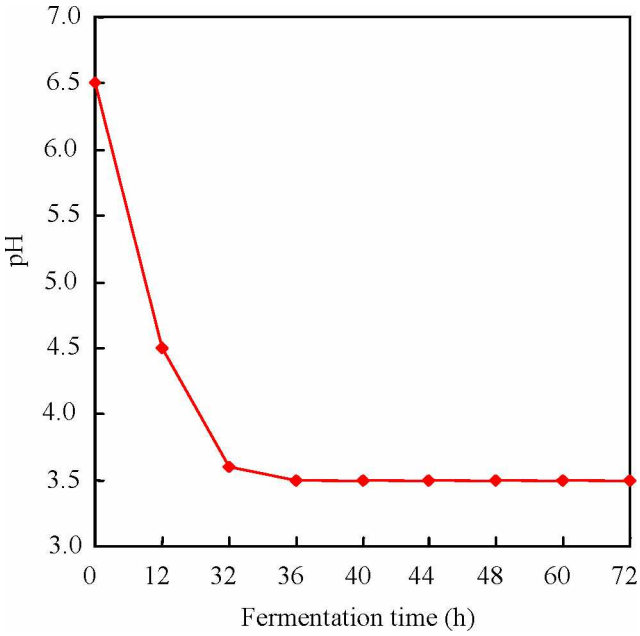

Figure 1. The change of pH in liquid-type yogurt fermented with *Lactobacillus casei* 911LC at 37°C for 72 h.

was determined after mixing the yogurt sample with 10 ml of hot distilled water (90°C) and titrating with 0.1 N NaOH using a 0.5% phenolphthalein indicator to an end point of faint pink color.

Free amino acid analysis

To determine free amino acid (FAA), 5 g of yogurt was mixed in 5 ml distilled water. Then 500 mg sulfosalicyclic acid was added to the mixture, after which the mixture was stored at 4°C for 1 h and centrifuged at 1,300×g for 15 min. The supernatant was filtered through a 0.45 μm filter paper and pre-treated by the method described by Lindroth and Mopper (1979). Determination of FAA by using high performance liquid chromatography (HPLC) was done by the modified method of Hodgin et al. (1983). Flow rate was 2 ml/min and two mobile phases were used: solvent A was 0.05 M sodium acetate (pH 6.3), and solvent B, methanol: THF (90:10, v/v). The linear gradient of solvent B was programmed at 5 levels as follows: initial starting at 20%, then increasing to 40% for 6 min, to 42% for 9 min, to 50% for 3 min and finally to 70% for 12 min. FAA was analyzed on an ODS-μ-Bondapak C column (3.9 mm×30 mm), and a HPLC (Waters, Plymouth, MN, USA) equipped with a RI detector was used. All quantitative analyses were performed by relating peak areas of individual FAA to those of external standard amino acids (Wako, Osaka, Japan). All samples were analyzed in triplicate.

Gel Electrophoresis

The SDS-PAGE analysis was carried out on a 15% separating gel containing acylamide and bisacrylamide (Schagger and Jagow, 1987). For identification, the molecular weight (Da) of standards used (Bio-Rad Laboratiories, Hercules, CA, USA) were as follows: phosphorylase b: 97,000, albumin: 66,000, ovalbumin: 45,000, carbonic anhydrase: 30,000, trypsin inhibitor: 20,100 and α-lactalbumin: 14,000. Yogurt samples (~20 g) were mixed with an equal volume of phosphate buffer which had 2% solution adjusted to pH 6.8, and the content was filtered (Whatman No. 42) to remove casein. The casein-free filtrate was filtered through a 0.45 μm membrane, and the filtrate was mixed with Laemmli buffer (Laemmli, 1970) containing SDS. This mixture was heated in a boiling water bath for 2 min. Samples were loaded in the wells of SDS gels, and electrophoresis was carried out at 30 mA for 2.5 to 3.0 h until the bromophenol blue dye reached the bottom of the gel. The gels were fixed in 10% TCA and silver stained to study the concentration and molecular mass of peptides present in the yogurt filtrate.

Sensory evaluation

For the sensory analysis, the fermented sample was diluted 4 times with distilled water. An eleven-person panel, semi-experienced in judging dairy products were recruited from faculty and graduate students in the Department of Food Science and Technology at Sejong University.

The intensity of yogurt flavor and bitterness was scored on a nine-point scale (1=none, 3=slight, 5=moderate, 7=strong and 9=very strong). A randomized, balanced, complete block design was used and all samples tested in duplicate.

Statistical analysis

Data from each experiment were analyzed by analysis of variance (ANOVA) using a SAS program (1990) and differences among treatments were determined by Duncan's multiple test at $p<0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

Changes in pH and titratable acidity

Changes in pH during the 72 h fermentation of yogurt are presented in Figure 1. The decrease in pH was dramatic during the first 32 h of the fermentation as pH 3.6 and plateaued thereafter. The titratable acidity increased dramatically up to 40 h fermentation, and plateaued between 40 to 60 h fermentation (Figure 2). At 72 h fermentation, the titratable acidity was 4.4%.

Joo (1987) has reported that pH decreased dramatically up to 12 h and kept steadily when *L. bulgaricus* IAM12090, *L. bulgaricus* IAM12091 and *L. bulgaricus* CH2 were used as a starter culture. Similar study (Soh, 1984) indicated that when *L. bulgaricus* CH2, *L. jugurti* 3048, and *L. helveticus* IAM1042 were starter cultures, the titratable acidity increased and plateaued at 48 h, while it increased steadily

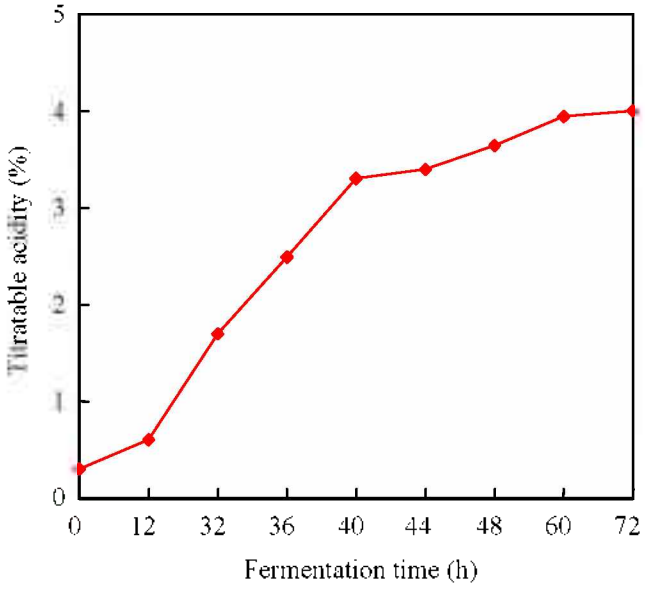

Figure 2. The change of titratable acidity in liquid-type yogurt fermented with *Lactobacillus casei* 911LC at 37°C for 72 h.

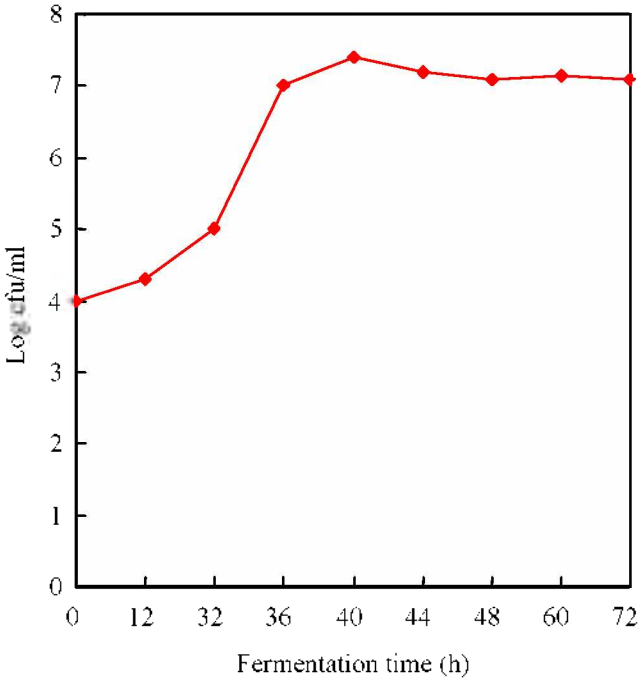

Figure 3. The change of lactic acid bacteria in liquid-type yogurt fermented with *Lactobacillus casei* 911LC at 37°C for 72 h.

up to 96 h with *L acidophilus* L54, *L. casei* YIT9018 and *L casei* 3012. That study showed *that L. bulgaricus L. jugurti*, and *L. helveticus* had more acid production than *L. acidophilus* and *L. casei*.

Changes in counts of *Lactobacillus casei* 911LC

Changes in the counts of *L. casei* 911LC during fermentation are presented in Figure 3. At 36 h fermentation, total counts reached 2.0×10^{7} cfu/ml at 36 h and plateaued up to 40 h, and decreased slowly during further incubation. However, counts were over 10^{7} cfu/ml at 72 h fermentation period, which was the regulation for the liquid-type yogurt.

Ha et al. (1992) has reported that the viable counts reached the highest at 24 h in most starter cultures. While it decreased dramatically after 24 h with *L. bulgaricus* and *L. helveticus*, *L. casei* and *L. acidophilus* kept high viable counts during long fermentation period.

Production of free amino acids

The production of free amino acids (FAA) for 72 h fermentation is shown in Table 1. The release of individual

Table 1. The change of free amino acid production in liquid-type yogurt fermented with *Lactobacillus casei* 911LC at 37°C for 72 h (μmol/ml)

Amino acid	Fermentation time (h)							
	12	32	36	40	44	48	60	72
Alanine	0.87	0.78	0.69	0.92	0.99	0.77	0.85	0.70
Asparagine	0.34	0.33	0.30	0.39	0.39	0.33	0.42	0.38
Glutamic acid	1.36	1.20	1.04	1.45	1.47	1.12	0.64	0.46
Lysine	1.34	2.28	1.83	1.77	1 68	1 41	1.09	0.56
Methionine	0.17	0.20	0.12	0.12	0.15	0.15	0.12	0.08
Serine	1.50	1.46	1.27	1.70	1.60	1.34	1.25	0.92
Tryptophan	0.02	0.03	0.03	0.03	0.02	0.02	0.04	0.03
Threonine	0.87	0.89	0.81	1.04	0.97	0.80	0.69	0.54
Valine	0.75	0.69	0.59	0.85	0.84	0.73	0.32	0.22
Aspartic acid*	0.60	0.70	0.60	0.88	0.81	0.63	0.11	0.03
Arginine*	0.20	0.17	0.14	0.16	0.27	0.13	0.29	0.25
Isoleucine*	0.33	0.34	0.27	0.35	0.45	0.30	0.17	0.12
Leucine*	1.25	1.32	1.03	1.40	1.49	1.11	0.87	0.64
Phenylalanine*	0.22	0.23	0.18	0.22	0.31	0.19	0.22	0.13
Tyrosine*	1.63	1.46	1.29	1.87	1.84	1.59	1.50	1.02
Total amino acids	11.45	12.08	10.19	13.15	13.28	10.62	8.58	6.08
Bitter amino acids	4.23	4.22	3.51	4.88	5.17	3.95.	3.16	2.19

* Represents bitter amino acids.

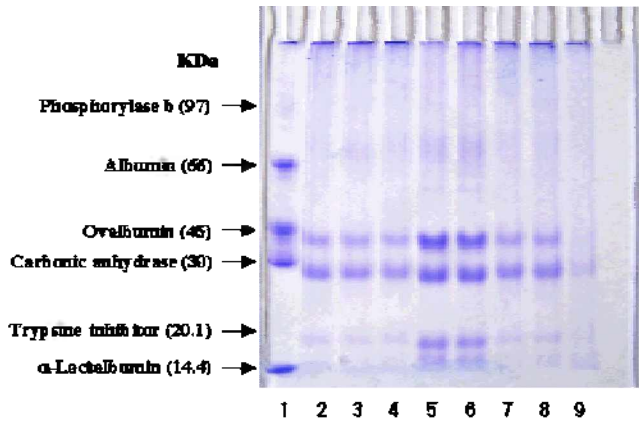

Figure 4. Electrophoretic patterns of casein on SDS 15% polyacrylamide gel of liquid-type yogurt fermented with *Lactobacillus casei* 911LC at 37°C for 72 h. 1: Low molecular marker, 2: 12 h, 3: 32 h, 4: 36 h, 5: 40 h, 6: 44 h, 7: 48 h, 8: 60 h and 9: 72 h.

free amino acids was the greatest mostly at 40-44 h fermentation and decreased slowly up to 72 h. More amounts of lysine, glutamic acid, leucine, tyrosine and serine were released, compared to other amino acids. The amount of total amino acids was the highest as 13.28 μmol/ml at 44 h. The amount of total bitter amino acids also the highest as 5.17 μmol/ml at 44 h. Among bitter amino acids, leucine and tyrosine were released more through the fermentation period.

Gel electrophoresis

No significant change in electrophoretic pattern was found during the 72 h fermentation (Figure 4). At 40 and 44 hr fermentation, the band was thicker than those of other periods.

In general, protein decomposition during the manufacture of yogurt is low, compared with that found in cheese. Thus, protein breakdown to water-soluble products, including peptides, amino acids and ammonia, amounts to 25-35% in hard cheese and to about 90% in some soft cheese varieties. The content of free amino acids in ready-to use yogurt amounts to about 1% of the total protein (Rasic and Kurmann, 1978). There is some controversy about the formation of peptides in yogurt. Hetzel (Rasic and Kurmann, 1978) established a considerable proportion of free peptides in yogurt, while other investigators did not find any significant amounts. In conclusion, the present study indicated that the optimum fermentation time for liquid-type yogurt using *Lactobacillus casei* 911LC was from 40 to 44 h.

Sensory analysis

The sensory attributes of liquid-type yogurt are shown in Table 2. The scores of yogurt flavor and bitterness

Table 2. The change of yogurt flavor and bitterness scores in liquid-type yogurt fermented with *Lactobacillus casei* 911LC at 37°C for 72 h[1]

Fermentation time (h)	Yogurt flavor[2]	Bitterness[2]
12	2.9[a]	2.5[a]
32	5.0[b]	5.3[b]
36	5.8[bc]	5.3[b]
40	6.2[bc]	5.5[b]
44	6.6[c]	6.1[c]
48	6.4[c]	5.6[bc]
60	6.9[c]	5.4[b]
72	6.5[c]	5.4[b]

[1] Means of 3 replications. Means not followed by the same letter in the same column differ significantly from one another ($p<0.05$).
[2] As the value of sensory scores increases from 1 to 9, the intensity of the sensory characteristics increases.

increased dramatically up to 32 h, and then slowly increased up to 36 h and plateaued thereafter. The intensity of yogurt flavor was 5.0 at 32 h fermentation, and reached the highest score at 44 h as 6.6. The bitterness score showed a similar trend to that of yogurt flavor score. The bitterness score was the highest at 44 h, which was in accordance to the highest amount of bitter amino acids.

In conclusion, the present study indicated that the optimum fermentation time for liquid-type yogurt using *Lactobacillus casei* 911LC was from 40 to 44 h.

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