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Physico-chemical Properties of *Lactobacillus casei* 00692 during Fermenting for Liquid-type Yogurt

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Abstract

This study was carried out to find the physico-chemical attributes of yogurt base with *Lactobacillus casei* 00692 during 72 hr fermentation at 37℃. The pH decreased up to 44 hr and plateaued thereafter, and the titratable acidity increased up to 40 hr. The number of lactic acid bacteria sharply increased with 1.0×10^7 cfu/mL up to 48 hr of fermentation and slowly increased thereafter. The free amino acids produced during the fermentation reached the maximum value at 40 hr and gradually decreased thereafter. In the result of electrophoresis, the band was the thickest at 44 hr and mostly disappeared at 72 hr fermentation. The present data showed that the range of optimum fermentation time for yogurt base using *Lactobacillus casei* 00692 was from 40 to 44 hr.

Key words : fermentation time, yogurt base, *Lactobacillus casei*

Introduction

Fermented dairy products have been a major part of the diet of people around the world. Numerous scientific papers and review articles (Dave and Shah, 1998; Hughes and Hoover, 1991; Kurmann and Rasic, 1993; Modler *et al.*, 1990) have been published on the health benefits associated with the consumption of fermented dairy products. Over the past decades, considerable study of probiotic organism (*Lactobacillus acidophilus* and *Bifodobacterium* spp.) has been developed in food, pharmaceutical, and feed products. The consumption of probiotic products has increased dramatically in most European, Asia-Pacific, and American countries, and more than 90 products containing *L. acidophilus*, bifidobacteria, or both are available in the worldwide market (International Dairy Federation, 1988). Some of the proposed health benefits are thought to be conferred by living bacteria contained in the products. Suggested minimum numbers of probiotic bacteria at consumption are 10^5 to 10^7 cfu/g (Kurmann and Rasic, 1991). However, recent market surveys have revealed that the viability of probiotic organisms in commercial preparations has been low (Anonymous, 1992; Schagger and Von Jagow, 1987).

Liquid-type yogurt has introduced in 8th century as named "Subuk yogurt" in Turkey (Rasic and Kurmann, 1978). Since in the middle of 1970s, liquid-type yogurt, which was introduced by Japan has gained widespread consumer acceptance in Korea. 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 Korean community.

Liquid-type yogurt is defined as follows: 3% of solid non fat contained and viable counts 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, 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 regions 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

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fermenting organisms, the type of milk, and the manufacturing process used (Tamine and Deeth, 1980).

The change pattern of proteolytic activity by lactic acid bacteria during fermentation is basically important. Proteinase activity was detected in several strains of lactobacilli and streptococci (Ezzat *et al.*, 1985; Zourari *et al.*, 1992).

Even though the observation of different kinds of single yogurt starter culture was generally applied, a little information is only 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* 00692 based on physico-chemical properties.

Materials and Methods

Starter Culture Preparation

Yogurt starter culture strain, *Lactobacillus casei* 00692, used in this study was obtained as lyophilized pure cultures from Culture Systems Inc. (Mishawaka, IN, USA). The medium, MRS Medium+0.02% cysteine, adjusted to pH 6.2~6.6. The lactic starter culture was inoculated and propagated three times in 10% (w/v) sterile reconstituted skim milk at 37℃.

The *Lactobacillus casei* 00692 subcultured was inoculated at 2.0% (v/v) into reconstituted NDM containing 16.5% skim milk, 4% glucose, and incubated at 37°C for 72 hr. During the fermentation, samples were taken at 0, 12, 32, 36, 40, 44, 48, 60, and 72 hr.

Microbiological Analyses

Lactic acid viable count was determined by BCP agar (Eiken Co., Tokyo, Japan). One gram of yogurt samples which were taken for each time was diluted with 9 mL of sterile peptone and water diluent. Subsequent serial dilutions of each sample were plated in triplicate and incubated at 37℃ for 72 hr. All samples were stored at 4℃ during the analysis.

Chemical Analyses

pH values of the yogurt samples were measured using a pH meter (Orion 900A, Boston, MA, USA). The titratable acidity was determined after mixing the yogurt sample with 10 mL of hot distilled water (90℃) and titrating with 0.1 N NaOH containing 0.5% phenolphthalein as 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 into the mixture, after which the mixture was stored at 4℃ for 1 hr and centrifuged at 1,300 × g for 15 min. The supernatant was filtered through a 0.45 μm membrane filter 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 Laboratories, 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 0.45μm membrane filter, 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 hr 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.

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 stated otherwise.

Results and Discussion

Changes in pH and Titratable Acidity

Changes in pH during the 72 hr fermentation of yogurt are presented in Fig. 1. The pH decreased dramatically during the first 32 hr of the fermentation as pH 5.2 and turned to a slow decrease thereafter. At 48 hr, pH was below 5.0. The titratable acidity increased dramatically up to 40 hr fermentation, and plateaued between 40 to 72 hr fermentation (Fig. 2). At 40 hr fermentation, the titratable acidity was 4.3%.

Joo (1987) has reported that pH decreased dramatically up to 12 hr 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 hr, while it increased steadily up to 96 hr with *L. acidophilus* L54, *L. casei* YIT9018, and L. casei 3012. That study showed that *L. bulgaricus*, *L. jugurti*, and *L. helveticus* produced more acid than *L. acidophilus* and *L. casei*.

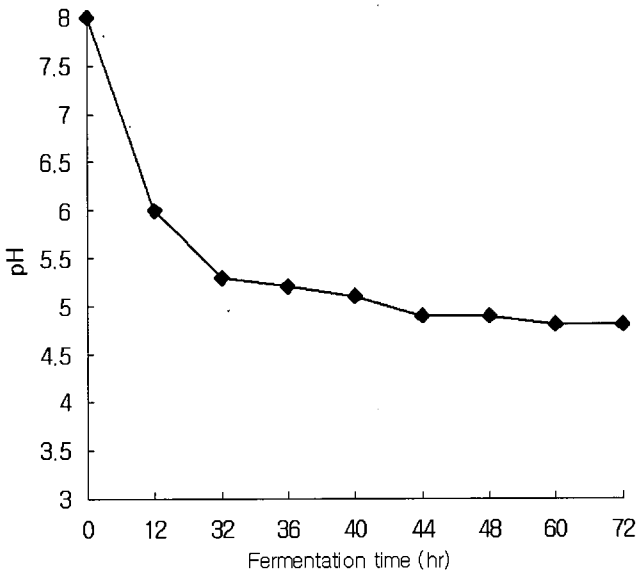

Fig. 1. Change of pH in yogurt base during fermentation at 37℃ for 72 hr.

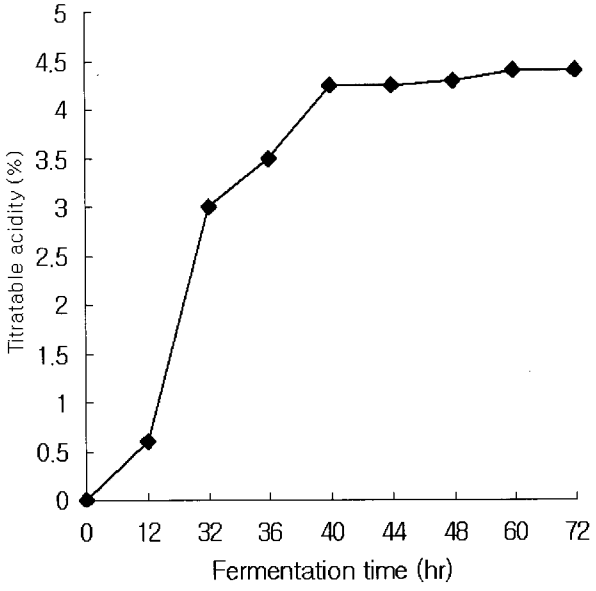

Fig. 2. Change of titratable acidity in yogurt base during fermentation at 37℃ for 72 hr.

Changes in Counts of *Lactobacillus casei* 00692

Changes in the counts of *L. casei* 00692 during fermentation are presented in Fig. 3. At 48 hr fermentation, total viable cell counts reached 1.0×10^7 cfu/mL and increased slowly to 4.8×10^7 cfu/mL at 60 hr, and decreased slowly during further incubation. However, counts were over 10^7 cfu/mL at 72 hr fermentation period, which was the regulation

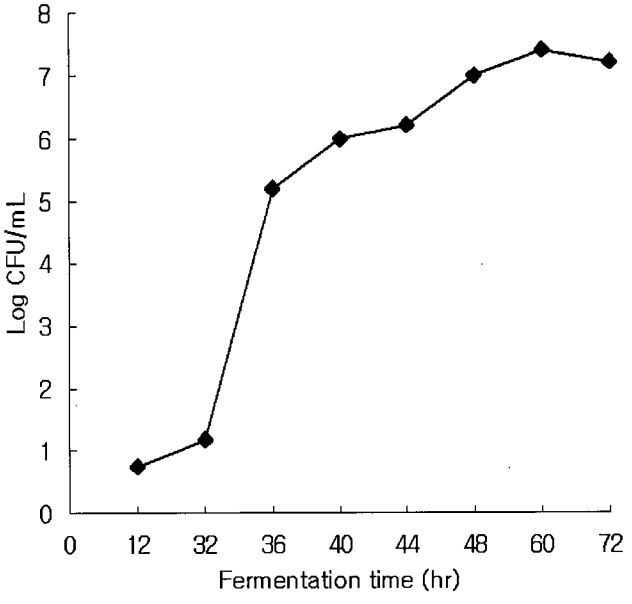

Fig. 3. Change of No of lactic acid bacteria in yogurt base during fermentation at 37℃ for 72 hr.

for the yogurt base.

Ha *et al.* (1992) has reported that the viable counts reached the highest at 24 hr in most starter cultures. While it decreased dramatically after 24 hr 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 hr fermentation is shown in Table 1. The release of individual free amino acids was the greatest mostly at 36~40 hr fermentation and decreased slowly up to 72 hr. More amounts of lysine and serine were released, compared to other amino acids. Among bitter amino acids, leucine and tyrosine were released more through the fermentation period.

Gel Electrophoresis

In the result of electrophoresis, no significant change in electrophoretic pattern was found during the 60 hr fermentation (Fig. 4). But the band was the thickest at 44 hr and mostly disappeared at 72 hr fermentation.

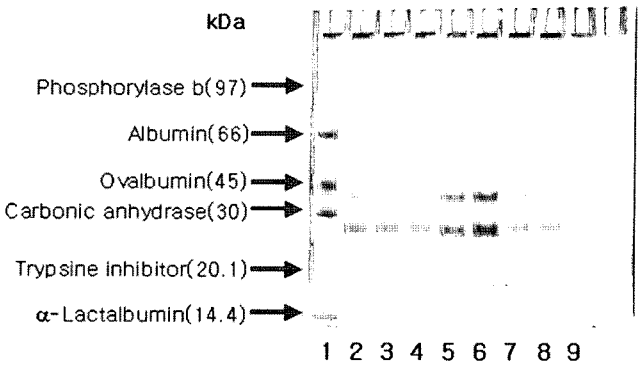

Fig 4. Electrophoretic patterns of casein on SDS 15% polyacrylamide gel of yogurt base during fermentation at 37℃ for 72 hr.
1: Low molecular marker, 2: 12 hr, 3: 32 hr, 4: 36 hr, 5: 40 hr, 6: 44 hr, 7: 48 hr, 8: 60 hr, and 9: 72 hr.

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 of 25~35% in hard cheese and 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).

Table 1. The production of free amino acid in yogurt base fermented with *Lactobacillus casei* 00692 at 37℃ for 72 hr.

(μmol/mL)

Amino acid	Fermentation time (hr)							
	12	32	36	40	44	48	60	72
Alanine	0.68	0.73	0.83	0.86	0.70	0.76	0.69	0.75
Asparagine	0.33	0.44	0.38	0.43	0.36	0.43	0.33	0.40
Glutamic acid	0.87	0.76	1.16	1.29	0.99	0.74	0.96	0.82
Lysine	1.45	1.82	2.00	2.27	2.05	0.99	1.68	1.42
Methionine	0.12	0.17	0.16	0.18	0.13	0.18	0.15	0.13
Serine	1.20	1.33	1.45	1.60	1.32	1.59	1.26	1.31
Tryptophan	0.02	0.03	0.02	0.03	0.03	0.02	0.02	0.02
Threonine	0.87	0.89	1.05	0.98	0.90	0.99	0.68	0.86
Valine	0.53	0.52	0.69	0.71	0.57	0.64	0.60	0.50
[1)]Aspartic acid	0.38	0.25	0.64	0.62	0.49	0.31	0.47	0.27
[1)]Arginine	0.14	0.20	0.16	0.18	0.16	0.17	0.16	0.20
[1)]Isoleucine	0.24	0.26	0.31	0.32	0.29	0.32	0.27	0.26
[1)]Leucine	0.93	1.04	1.21	1.27	1.11	1.15	1.09	1.02
[1)]Phenylalanine	0.17	0.22	0.20	0.21	0.20	0.22	0.19	0.20
[1)]Tyrosine	1.22	1.41	1.48	1.59	1.32	1.32	1.32	1.36
Total amino acids	9.15	10.07	11.74	12.54	10.62	9.83	9.87	9.52
Bitter amino acids	3.08	3.38	4.00	4.19	3.57	3.49	3.50	3.31

[1)]Represents bitter amino acids

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 was carried out to find the optimum fermentation time of *L. casei* 00692, since the optimum fermentation time of yogurt base mainly depends on the kind of starter culture. When the physico-chemical attributes of yogurt base with *Lactobacillus casei* 00692 during 72 hr fermentation at 37℃ were examined, the pH decreased up to 44 hr and the titratable acidity increased up to 40 hr. The growth of lactic acid bacteria sharply increased to 1.0×10^7 cfu/mL at 48 hr of fermentation. The free amino acids produced during the fermentation reached the maximum value at 40 hr. The results indicated that the range of optimum fermentation time for yogurt base using *Lactobacillus casei* 00692 was from 40 to 44 hr.

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