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Changes of Physico-Chemical Properties in Liquid-type Yogurt with *Lactobacillus casei* 00692 duirng Fermentation

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INTRODUCTION

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., 1983), the amount of solids (Nakanishi and Yanaji, 1983)[1], and kinds of starter and etc. (Lee et al., 1994).

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)[2].

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* 00692 based on physico-chemical properties.

MATERIALS AND METHODS

Starter Culture Preparation

Yogurt starter culture strain, *Lactobacillus casei* 00692, used in this study were obtained as lyophilized pure cultures from Korea Food Rearch Institute. The lactic starter culture was inoculated and propagated three times in 10% (w/v) sterile reconstituted skim milk at 37℃[3].

The *Lactobacillus casei* 00692 was inoculated at 2.0% (v/v) into reconstituted NDM containing 16.5% skim milk, 4% glucose, and incubated at 37℃ 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 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℃ for 72 hr.

Chemical analyses

pH values of the yogurt samples were measured using a pH meter (Model USA) after calibration with fresh pHs 4.0 and 7.0 standard buffers.

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℃ for 1hr and centrifuged at 1,300 × g for 15 min. The supernatant was filtered through a 0.45 m 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).

RESULTS AND DISCUSSION

Changes in pH

Changes in pH during the 72 hr fermentation of yogurt are presented in Fig. 1. The decrease in pH was dramatic during the first 32 hr of the fermentation as pH 5.2 and plateaued thereafter.

Changes in counts of *Lactobacillus casei* 00692

Changes in the counts of *L. casei* 00692 during fermentation are presented in Fig. 2. At 40 hr fermentation, total counts reached 2.0×10^6 cfu/mL and slowly increased thereafter. However, counts were over 10^7 cfu/mL at 72 hr fermentation period, which was the regulation for the liquid-type yogurt. Production of lactic acid is the most important chemical process, which occurs during yogurt manufacture. The lactic acid helps destabilizing the casein micelle and this leads to coagulation of the milk protein and formation of the yogurt gel. The lactic acid also gives the sharp acid taste to yogurt and contributes to the typical 'aromatic' flavor[4].

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 40 hr fermentation and decreased slowly up to 72 hr. More amounts of lysine, serine, leucine and tyrosine were released, compared to other amino acids. The amount of total amino acids was the highest as 12.54 μmol/mL at 40 hr. The amount of total bitter amino

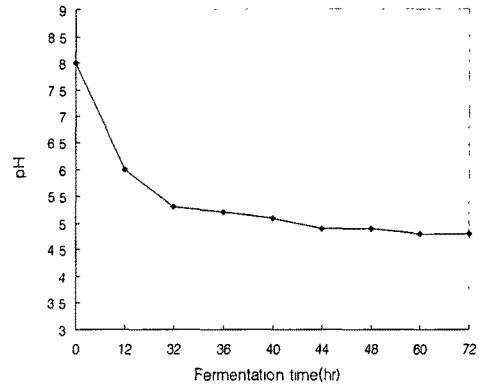

Fig. 1. Changes of pH in liquid-type yogurt during the fermentation at 37℃ for 72hrs.

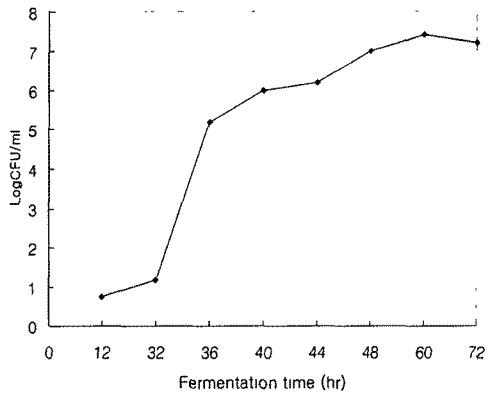

Fig. 2. Changes of lactic acid bacteria counts in liquid-type yogurt during the fermentation at 37℃ for 72hrs.

Table 1. The change of free amino acid production in liquid-type yogurt 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
*Aspartic acid	0.38	0.25	0.64	0.62	0.49	0.31	0.47	0.27
*Arginine	0.14	0.20	0.16	0.18	0.16	0.17	0.16	0.20
*Isoleucine	0.24	0.26	0.31	0.32	0.29	0.32	0.27	0.26
*Leucine	0.93	1.04	1.21	1.27	1.11	1.15	1.09	1.02
*Phenylalanine	0.17	0.22	0.20	0.21	0.20	0.22	0.19	0.20
*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	3.45	3.38	4	4.19	3.57	3.49	3.5	3.31

*Represents bitter amino acids

acids also the highest as 4.19 μmol/mL at 40 hr. Among bitter amino acids, leucine and tyrosine were release more through the fermentation period.

SUMMARY

This study was carried out to find the physico-chemical attributes for liquid-type yogurt with *Lactobacillus casei* 911LC during 72 hr fermentation at 37℃. The pH decreased up to 32 hr and plauteaued thereafter. The growth of lactic acid bacteria sharply increased with 2.0×10^6 cfu/mL up to 40 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. The present data showed that the range of optimum fermentation time for liquid-type yogurt using *Lactobacillus casei* 00692 was from 40 to 44 hr.

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