International Journal of Agricultural Technology 2022Vol. 18(1):393-400
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Growth and survival of lactic acid bacteria during the fermentation of durian yogurt

Waritchon, N.* **and Kunlaporn, P.**

Faculty of Agricultural Technology, Rambhai Barni Rajabhat University, Thailand.

Waritchon, N. and Kunlaporn, P. (2022). Growth and survival of lactic acid bacteria during the fermentation of durian yogurt. International Journal of Agricultural Technology 18(1):393-400.

Abstract The behaviors of lactic acid bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophillus*, during the fermentation of durian yogurt was investigated. Ripened pulp of durain was found to support the growth of the both of *L.bulgaricus* and *S. thermophillus*. The pH values ranged between 4.58-4.68, TSS 24.0-26.0%, and acidity (as lactic) 0.95-1.21% in yogurt after 24 hr of fermentation. The viable populations of lactic acid bacteria increased rapidly in the fermented yogurt after 4 hr to 12 hr fermentation with numbers averaged $1.22x10^7$ cfu/g *L.bulgaricus* and $1.85x10^7$ cfu/g *S. thermophillus*, their population stabilized after 12 hr to 24 hr of fermentation with numbers averaged $1.80x10^7$ cfu/g *L.bulgaricus* and $1.12x10^7$ cfu/g *S. thermophillus* after 24 hr of fermentation. The optimum time between 10-12 hr had the optimum characteristics of yogurt with pH 4.61-4.73, TSS 26.5%, lactic acid 0.92-1.10% and lactic bacteria count of 1.20-$1.50x10^7$cfu/g.

Keywords: Durian, Yogurt, Lactic acid bacteria, Lactic acid fermentation

Introduction

Durian (*Durio zibethinus*) is one of the most important tropical fruit crops grown in Thailand. The edible portion of the fruit, known as the aril and usually referred to as the "flesh" or "pulp", only accounts for about 15-30% of the mass of the entire fruit . The fruit's texture is similar to custard or yogurt, its distinct flavor and unique taste (Hokputsa *et al.*, 2004). Durian fruit contains a high amount of sugar, vitamin C, potassium, and the serotonergic amino acid tryptophan, and is a good source of carbohydrates, proteins, and fats (Husin *at al.*, 2018). In Malaysia and Indonesia, underutilised durian pulp is fermented (spontaneous and uncontrolled) to a product known as Tempoyak, it is widely used as seasoning in cooking. Khalil *et al*. (2018) and Ahmad *et al*. (2018) recently demonstrated the potential of Tempoyak as a source of probiotics. Therefore, this fruit is a good substrate for lactic acid bacteria in a yogurt-type fermentation, which the potential of science and the bounty of nature, a rich custard highly flavored with almonds gives the best general idea of it.

***Corresponding Author:** Waritchon, N.; **Email:** waritchon_n@hotmail.com

Yogurt is produced by adding two starter cultures, *Lactobacillus bulgaricus* and *Streptococcus thermophillus* to milk. During the fermentation, hydrolysis of the milk proteins occurs, the pH drops, the viscosity increases, and bacteria metabolites are produced that contribute to taste and possibly to the health promoting properties of yogurt. The fermentation of durian with lactic acid bacteria has been studied to develop for new product of yogurt-type (Ninlanon, 2007), but no information is available on the growth of the species (*L. bulgaricus* and *S. thermophillus*). Therefore, the growth and survival of lactic acid bacteria, and acid production during the fermentation of durian yogurt was investigated.

Materials and methods

Cultures

Mixed cultures of *L. bulgaricus* and *S. thermophillus* from Chr. Hansen's laboratory were propagated in GYP medium, sterilized at 121 ℃ for 15 min, at 43 ℃ incubate temperature for 24 hr and, then, used in all experiments.

Preparation of yogurt

Batch of yogurt were prepared in sterilized glass bottles, containing 30% ripened pulp of durian. The pulps was blended 5 min with pasteurized cow's milk. The mixture supplemented with 5% sucrose and 5% skim milk (Ninlanon, 2007). The resultant slurry was filtered through a double-layered cheesecloth, and then, pasteurized at 80 ℃ for 15 min, cooled and inoculated with 5% lactic cultures.

Conditions

When fermentation was performed, 100 ml of sterile slurry was placed in a glass bottle, it was inoculated with 0.5 ml of an inoculum of inocula of lactic acid bacteria. The initial population of each organism in the slurry was between 3-4 log cfu/ml^{-1}. Inoculate slurry was inoculated at 43 ℃ for 24 hr. During the period, samples were taken to determine the pH and titratable acidity of and the numbers of lactic acid bacteria in the yogurt.

Microbiological and chemical analyzes

L. bulgaricus and *S. thermophillus*were enumerated using MRS agar (Difco). One milliliter of appropriate serial dilutions of each sample were pour-plated onto the appropriate media. After 24 hr of incubation at 43 ℃, the colonies that appeared on the plates were counted and the cfu/ml were calculated.

The titratable acidity (TA) was determined by the AOAC method and expressed as the percentage of lactic acid (AOAC, 1984). The pH values were determined using a pH meter while total soluble solid (TSS) were determined using a refractometer (Atago, Japan).

Statistical analysis

The mean values and the standard deviations were calculated from the data obtained with triplicate trials. Means were separated using the Duncan multiple range test.

Results

Growth of lactic acid bacteria during fermentation

The growth of lactic acid bacteria shows in Figure 1. During the first 4 hr of incubation, the numbers of *L. bulgaricus* and *S. thermophillus* both did not show any significant growth, when lactic acid bacteria were included in the starter culture increased about $1.7x10^5$ cfu/ml during fermentation time. After 4 hr of incubation, the numbers of *S. thermophillus* were significantly higher ($P<0.05$) than the numbers of *L. bulgaricus*, their growth exhibited the highest by about $1.85x10^7$cfu/ml at 12 hr, thereafter, its numbers declined, the lowest final numbers after 24 hr of fermentation. While the numbers of *L. bulgaricus* were significantly higher than the numbers of *S. thermophillus* after incubation for 24 hr, the population highest increased about $2.3x10^7$ cfu/ml at 20 hr of incubation. The numbers of both lactic acid bacteria decreased on the after 12 hr of incubation, which final numbers at the end of fermentation were no significantly difference with numbers averaged $1.80x10^7$ cfu/ml of *L. bulgaricus* and $1.12x10^7$ cfu/ml of *S. thermophillus*.

Changes of TA, pH and TSS during fermentation

Changes in the pH and TA during fermentation of yogurt inoculated with lactic acid bacteria are summarized in Figure 2. TA increased and the pH decreased as the fermentation time increased. TA increasing from an initial 0.04% to 1.40% after 24 hr, while the pH values decreased ranging from 6.10 to 4.32 at the end of fermentation which corresponds to decreasing of TSS from 27.50 to 26.30%, during the same period of time (Figure 3).

As there is a correlation between the increasing of cell numbers, TA, and pH values (Figure 4-5), post-process acid production is likely the main factor causing the reductions of the numbers of lactic acid bacteria in the fermented slurry-durian.

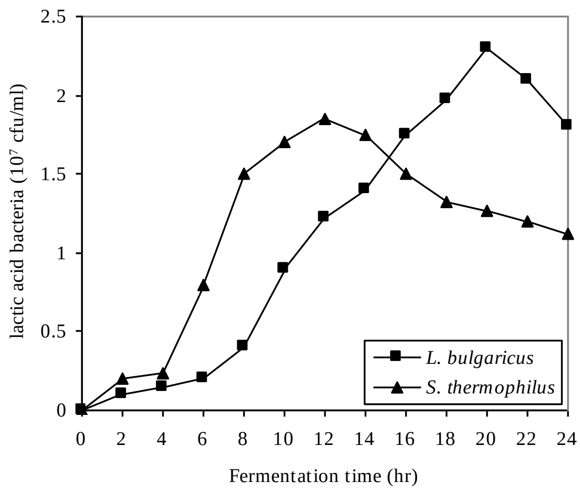

Figure 1. Growth of *L. bulgaricus* and *S. thermophillus*in durian yogurt with 30% ripened pulp-durian mixture supplemented 5% sucrose and 5% skim milk within 24 hr fermentation time at 43°C

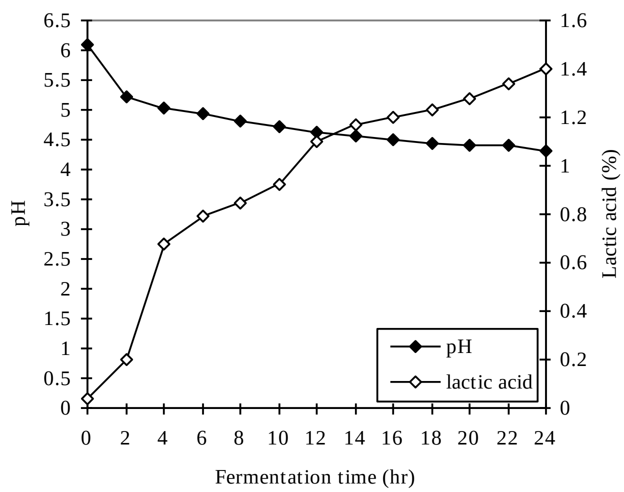

Figure 2. The relationship between lactic acid production and pH values in durian yogurt with 30% ripened pulp-durian mixture supplemented 5% sucrose and 5% skim milk within 24 hr fermentation time at 43°C

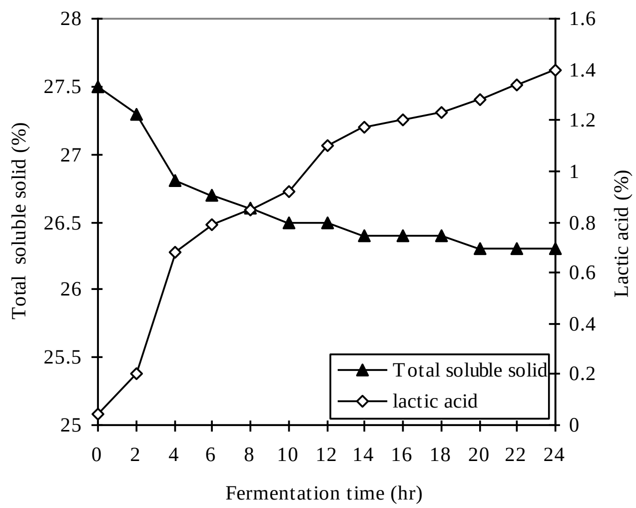

Figure 3. The relationship between total soluble solid and lactic acid production in durian yogurt with 30% ripened pulp-durian mixture supplemented 5% sucrose and 5% skim milk within 24 hr fermentation time at 43^{o}C

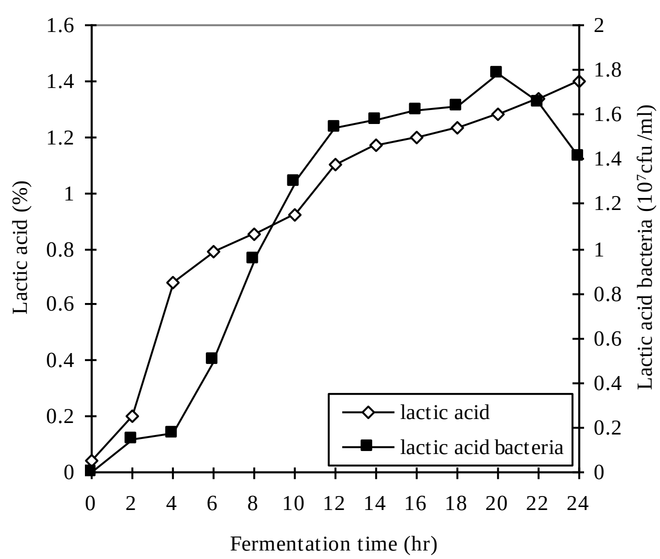

Figure 4. The relationship between cell numbers of lactic acid bacteria and lactic acid production in durian yogurt with 30% ripened pulp-durian mixture supplemented 5% sucrose and 5% skim milk within 24 hr fermentation time at 43^{o}C

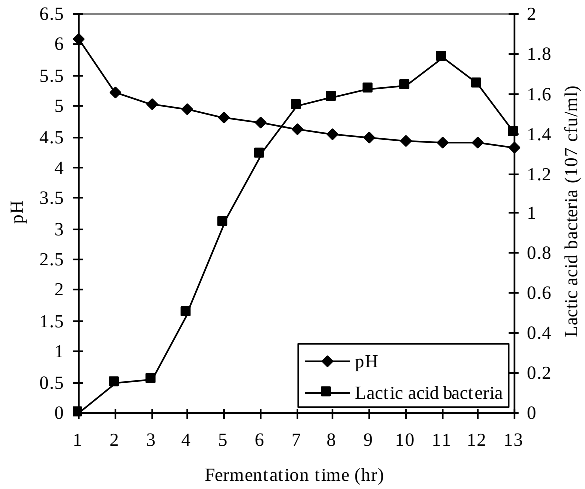

Figure 5. The relationship between cell numbers of lactic acid bacteria and pH values in durian yogurt with 30% ripened pulp-durian mixture supplemented 5% sucrose and 5% skim milk within 24 hr fermentation time at 43 °C

Discussion

The growth patterns of the yogurt starters in milk, the streptococci initiate the milk fermentation and lactobacilli contribute to acidification later in the incubation (Tamime and Robinson, 2007). This study showed that the pattern also occured in slurry-durian fermentation. Initially growth of *L. bulgaricus* increased slowly in slurry-durian. It is expected that *L. bulgaricus* strain did not grow well in durian yogurt, that may result from its high sulfur content in the nature of durian fruit. Organosulfur compounds have been found to inhibit the activity of the cyclooxygenase, lipoxygenase, bacteria and fungi (Kramer, 2010). Similar results had also been reported by Murti *et al*. (1993) and Farnwort *et al*. (2007), the most *L. delbrueckii* sub sp. *bulgaricus* strains did not grow in soy beverages.

Sucrose and skim milk added to the slurry-durian substrates in this study may have contributed to this faster growth. In some yogurt production, supplements such as whey powder, whey protein concentrates or acid casein hydrolysates are added to reduce the time required for fermentation with *L. delbrueckii*, because they provide amino acid and carbohydrates to support the growth of the organism (Dave and Shah, 1998). Growth of probiotic bacteria in cows' milk and other yogurt may be dependent on the liberation of amino acids by other bacteria (Farnwort *et al*., 2007).

Moreover, the growth of the added bacteria was delayed for 2 to 4 hr after the start of the fermentation. Presumably this time was needed to generate enough amino acids to support growth. Farnwort *et al.* (2007) described in general of cows'milk often dose not support extensive growth of bacteria because of the lack of free amino acids. Yogurt starter culture therefore contain bacteria with proteolytic activity, such as *L. delbrueckii* subsp. *bulgaricus*, that break down protein to produce amino acids that support the growth of non proteolytic bacteria (Malaka *et al*., 2020). In tradition yogurt, *S. thermophillus* is generally the beneficiary of this proteolysis, but in this results the probiotics could have benefited from it. It is confirmed the data in the figure which showed that *L. bulgaricus*is well grow in slurry-durian substrates after 12 hr fermentation, while growth of *S. thermophillus* stopped after 12 during fermentation.

However, other factors could be involved such as reduction of the free oxygen level as a result of lactic acid bacteria growth, or a drop in pH to level more favourable for the probiotics (Farnwort *et al.,* 2007), therefore, after 4 hr incubation, growth of the organism improved significantly. It could be due to the faster drop in pH which favourable to the growth of both lactic acid bacteria. It had been stated that the so called minimum therapeutic level of viable probiotic microorganisms should be at least 10^6 CFU/g of viable cells throughout the product shelf-life (Terpou *et al.*, 2019). Therefore, the fermented durian product containing a mixed culture, the number of lactic acid bacteria might be sufficient to impart beneficial effects. This study showed that when the lactic acid bacteria population remained 1.4 x 10^7 cfu/ml. The results indicated that durian yogurt has possibility to do further research on its feasibility to do health food product.

References

Ahmad, A., Yap, W. B., Kofli, N. T. and Ghazali, A. R. (2018). Probiotic potentials of *Lactobacillus plantarum* isolated from fermented durian (Tempoyak), a Malaysian traditional condiment. Food Science and Nutrition, 6:1370-1377.

AOAC (1984). Official Methods of Analysis. 14th ed. The Association of Official Analytical Chemists, Virginia.

Dave, R. I. and Shah, N. P. (1998). Ingredient supplementation effects on viability of probiotic bacteria in yogurt. Journal of Dairy Science, 81:2804-2816.

Farnwort, E. R., Mainvilla, I., Desjardins, M. P., Gardner, N., Fliss, I. and Champagne, C. (2007). Growth of probiotic bacteria and bifidobacteria in a soy yogurt formation. International Journal of Food Microbiology, 116:174-181.

Hokputsa, S., Gerddit, W., Pongsamart, S., Inngjerdingen, K., Heinze, T., Koschella, A., Harding, S. E. and Paulsen, B. S. (2004). Water-soluble polysaccharides with pharmaceutical importance from durian rinds (*Durio-zibethinus* Murr.) : isolation, fractionation, characterization and bioactivity. Carbohydrate Polymers, 56:471-481.

Husin, N. A., Rahman, S., Karunakaran, R. and Bhore, S. J. (2018). A review on the nutritional, medicinal, molecular and genome attributes of Durian (*Durio zibethinus* L.), the King of fruits in Malaysia. Bioinformation, 14:265-270.

Khalil, E. S., Manap, M. Y. A., Mustafa, S., Alhelli, A. M. and Shokryazdan, P. (2018). Probiotic properties of exopolysaccharide-producing lactobacillus strains isolated from tempoyak. Molecules, 23:398.

Kramer, S. R. (2010). Benefits of durian. Retrieved from http://www.healthy-communications.com/durianbenefits.htm

Malaka, R., Maruddin, F., Dwyana, Z. and Vargas, M. V. (2020). Assessment of exopolysaccharide production by *Lactobacillus delbrueckii* subsp. *bulgaricus* ropy strain in different substrate media. Food Science & Nutrition, 8:1657-1664.

Murti, T. W., Bouillanne, C., Landon, M. and Desmazeaud, M. J. (1993). Bacterial growth and volatile compounds in yogurt-type products from soy-milk containing *Bifidobacterium* sub sp. Journal of Food Science, 58:153-157.

Ninlanon, W. (2007). Development of a yogurt-type from durian. The Proceeding of 6^{th} Naresuan University Annual Conference, Agriculture Natural Resources and Environment, 119 p**.**

Tamime, A. Y. and Robinson, R. K. (2007). Yogurt science and technology. Science and Technology Woodhead Publishing Series in Food Science, Technology and Nutrition, pp. 608-645.

Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A. and Kopsahelis, N. (2019). Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. Nutrients, 11:1591.

(Received: 29 August 2021, accepted: 20 December 2021)