Production of sweet corn milk yogurt using *Lactobacillus casei*

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Abstract. This study aimed to produce a new functional fermented food product from sweet corn milk yogurt using Lactobacillus casei. The suitable conditions for lactic acid fermentation in sweet corn milk were investigated, including initial bacterial concentration, ratio of sweet corn milk and cow milk, sugar concentration, fermentation time and temperature. The results showed that the appropriate conditions for sweet corn milk yogurt production as follow: initial bacterial concentration was 106 cells/mL, fermentation time was 12 h at 37°C and sweet corn milk was fortified with cow milk at a ratio of 2:8 and 12% of sugar. The sweet corn milk yogurt was slightly yellow, smooth texture and thick body with the aroma of corn and acidic smell. The product remained acceptable taste and texture within 4 weeks at 0°C and 2 weeks at 5°C.

1 Introduction

Yogurts are important elements of the human diet, due to their high nutritional value and their appealing sensory properties [1]. Yogurt is one of the most popular fermented dairy products and its consumption is increasing worldwide [2]. According to the Euromonitor database, the yogurt production in 2015 reached 27.7 million metric tons, a 1.2-fold increase compared with the yield in 2010 [3]. Yogurt appeared a long time ago and principally made from cow's milk by acid lactic fermentation [4]. Yogurt has the source of proteins, vitamins, and minerals (especially calcium, vitamin B2, B6, B12) [5-6]. Yogurt is defined as the product being manufactured from milk (with or without the addition of some natural derivative of milk, such as skim milk powder, whey concentrates, caseinates, cream with a gel structure that results from the coagulation of the milk proteins, due to the lactic acid secreted by defined species of bacterial cultures [7].

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Considerable knowledge has been accumulated on the lactic acid bacteria (LAB) that affect the aroma and flavor of yogurt [8]. The predominant organisms in these starter cultures are lactic acid bacteria, for example, Lactococcus lactis, Lactobacillus species, Streptococcus thermophilus, Bifidobacterium species, and Leuconostoc species [9]. Lactobacillus casei is a strain with satisfying technological characteristics and therefore has numerous applications in the production of food products such as cheese [10], sausages [11], fermented milks [12], and yogurts [13, 14].

Nowadays, fresh fruits, fruit juices or cereals were added in yogurt to vary the food resources and improve the nutritional value of yogurt, such as carrot [6], banana [15], mango, and papaya [16]. Combining the intake of yogurt and fruit could provide probiotics, prebiotics, high-quality protein, important fatty acids, and a mixture of vitamins and minerals that have the potential to exert synergistic effects on health [17]. Corn is being cultivated worldwide for its demand as a high energy, micronutrient rich value-added food and it is being used by developing countries for food production while developed countries use it for industrial purposes [18, 19]. Sweet corn contains 5-6% sugar, 10-11% starch, 3% water-soluble polysaccharides and 70% water, besides moderate levels of protein and vitamin (yellow varieties) and potassium [20]. Sweet corn contains significant amount of lutein, zeaxanthin, and other carotenoids [21]. Sweet corn was reported to contain 75.7% moisture, 6.8 mg/100 g vitamin C, 2.0 mg/100 g calcium, 37 mg/100 g magnesium, 15.2 mg/100 g of sodium on fresh matter basis [22]. Therefore, using corn milk as the raw material for yogurt making can bring healthy benefits and can exploit the potential market for corn milk. Thus, this study aimed to evaluate the effects of starter concentration, ratio between corn milk and cow milk, amounts of adding sugar, temperature and time fermentation, and storage time of sweet corn milk yogurt.

2 Materials and methods

2.1 Chemical and cultures

MRS agar (De Man, Rogosa and Sharpe, Merck), MRS broth (Merck), yeast extract, beef extract, peptone, glucose, K2HPO4, diammonium citrate, MnSO4, MgSO4, Tween 80, sodium acetate, CaCO3. Sweet corn was bought from local markets in Can Tho city, Vietnam. Fresh milk was from Vinamilk (Vietnam). Five strains of Lactobacillus casei (FBLc01, FBLc02, FBLc03, FBLc01, and FBLc05) were stored at Biotechnology Research and Development Institte, Can Tho University, Vietnam..

2.2 Fermentation procedures

The production of sweet corn milk yogurt and experiments in this study were described in Fig. 1. The mixtures of cow milk and sweet corn milk (experiment 2, section 2.4) were boiled at 100°C and continuosly stirred in 10 minutes. Then, it was cooled to 30-37°C and inoculated with different strains of L. casei and initial cell densities (experiment 1, section 2.3). The fermentation processes were carried out at different temperatures and times (experiment 3, section 2.5). The final products were storaged at different temperatures (experiment 4, section 2.6) to evaluate the change of sweet corn milk yogurt characteristics (pH, total acid, cell number, and sensory) in 4 weeks.

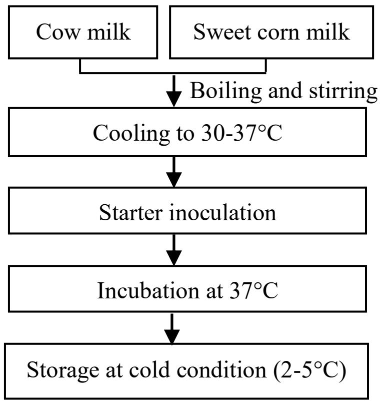

Fig. 1. The procedure to prepare yogurt from sweet corn milk

2.3 Selection of L. casei strain and inoculum level

To identify the best performed L. casei strain and suitable inoculum level for sweet corn milk fermentation. Five strains of L. casei were grown in MRS broth to increase cell concentration and diluted to the concentration of 104, 106, and 108 cells/mL. Then added to sweet corn milk mixture (corn/cow milk ratio of 2:8 and 3% of sugar) and incubated for 12 h at 37°C. The final fermentation products were evaluated the pH, total acidity, cell number, and sensory.

2.4 Effect of the ratio of corn/cow milk and sugar concentration

To indicate the suitable ratio between corn juice, milk and sugar adding for sweet corn yogurt. Cell density was determined in section 2.3. Different ratios of corn milk and cow milk (1:9, 2:8, 3:7, 4:6, and 5:5), and percentages of adding sugar (4, 8, 12, and 16% w/v) were investigated. The final products were analysed the pH value, total acidity, cell number, and sensory.

2.5 Effect of fermentation temperature and time

To determine the favorable fermentation temperature and time for sweet corn milk yogurt production. Used starter and sweet corn milk were the results of section 2.3 and 2.4. However, the unit experiments will be incubated at different temperatures (30°C, 37°C, and 40°C) and time (6, 9, 12, and 15 h). The pH, total acidity, cell number, and sensory of final products were evaluated as previous experiment.

2.6 Effect of the storage condition of sweet corn milk yogurt

To determine the suitable storage conditions for sweet corn yogurt. Sweet corn milk yogurt was stored at 0°C and 5°C in 4 weeks for evaluation of product characteristics.

2.7 Fermentation parameters and data analysis

The pH value was measured using a pH meter (pH Mettler Toledo 320). Titratable acidity was analysed with NaOH 0.1 N and phenolphthalein as an indicator [23]. Cell number was estimated by the method of plate counts using MRS medium [24]. The sensory score was performed with 10 people using description scale (0-5) of texture, aroma, acidity, and colour followed the Vietnamese Standard 3215:79 [25]. Experimental data were processed statistically by Statgraphics Centurion XV.I software (Statpoint Technologies Inc., USA) and charted by Microsoft Excel 2013 (Microsoft Corporation, USA).

3 Result and discussion

3.1 Fermentation ability of L. casei and inoculum cell density

The pH and total acidity (TA) values are shown in Table 1. pH values fluctuated from 4.30 ± 0.11 to 6.59 ± 0.21 and TA values were from 0.29 ± 0.03% to 0.79 ± 0.01%. Cell numbers were varied from 9.10 ± 0.34 log cells/mL to 11.49 ± 0.45 log cells/mL after 12 h of incubation. All three criteria had a statistical difference. Generally, with the treatment had the same type of starter and the different cell density, the higher cell density was, the lower pH was, and TA value was high.

Table 1. Fermentation of sweet corn milk yogurt with different *L. casei* strains and cell densities.

Strain	Cell number (cells/mL)	pH	Total acidity (%)	Cell number (log cells/mL)	Sensory score
FBLc01	10^8	4.69 ± 0.03^{bc}	0.68 ± 0.01^{bc}	11.11 ± 0.52^{ab}	14.4 ± 0.71^{c}
FBLc01	10^6	4.90 ± 0.01^{cd}	0.64 ± 0.03^{cd}	10.38 ± 1.03^{cd}	15.5 ± 1.02^{abc}
FBLc01	10^4	6.12 ± 0.07^{g}	0.41 ± 0.01^{hi}	10.24 ± 0.84^{cd}	17.5 ± 0.32^{ab}
FBLc02	10^8	4.56 ± 0.04^{ab}	0.61 ± 0.02^{b}	10.09 ± 0.52^{d}	16.1 ± 0.98^{abc}
FBLc02	10^6	5.85 ± 0.11^{g}	0.53 ± 0.04^{hg}	9.62 ± 0.71^{e}	14.7 ± 1.47^{abc}
FBLc02	10^4	6.59 ± 0.21^{h}	0.29 ± 0.03^{j}	9.10 ± 0.34^{f}	16.7 ± 0.63^{abc}
FBLc03	10^8	4.30 ± 0.11^{a}	0.79 ± 0.01^{a}	11.49 ± 0.45^{a}	13.0 ± 0.93^{c}
FBLc03	10^6	4.63 ± 0.08^{bc}	0.69 ± 0.01^{b}	11.18 ± 0.54^{ab}	18.1 ± 1.16^{a}
FBLc03	10^4	5.95 ± 0.13^{g}	0.44 ± 0.00^{ghi}	10.38 ± 1.15^{cd}	18.1 ± 1.04^{a}
FBLc04	10^8	5.19 ± 0.16^{ef}	0.57 ± 0.01^{ef}	10.97 ± 0.63^{b}	15.3 ± 0.38^{abc}
FBLc04	10^6	5.83 ± 0.09^{g}	0.46 ± 0.01^{g}	10.58 ± 0.46^{c}	18.1 ± 0.71^{a}
FBLc04	10^4	5.83 ± 0.12^{g}	0.39 ± 0.00^{i}	10.20 ± 1.41^{cd}	17.7 ± 0.82^{ab}
FBLc05	10^8	5.03 ± 0.14^{de}	0.61 ± 0.05^{e}	10.49 ± 0.76^{c}	16.4 ± 0.91^{abc}
FBLc05	10^6	5.36 ± 0.07^{f}	0.53 ± 0.01^{f}	10.30 ± 0.63^{cd}	17.6 ± 1.07^{ab}
FBLc05	10^4	6.52 ± 0.21^{h}	0.33 ± 0.00^{j}	10.21 ± 0.84^{cd}	15.5 ± 0.61^{abc}
Coefficient of variation (%)		6.12	12.21	11.09	14.16

Note: Values in the table were the average values of triplications. Difference values of statistics only had meaning in the column; the average values with the same letter were not significant at 95% probability. The suitable pH for yogurt is 4.1-4.6 (at this pH range the yogurt was firmer) and TA is 0.7-0.9% [26]. Therefore, treatments using L. casei FBLc01, FBLc02 with cell number of 108 cells/mL and L. casei FBLc03 with cell numbers of 106 and 108 cells/mL had suitable pH and TA values. However, when comparing the cell number values, treatments using L. casei FBLc01-108 cells/mL, L. casei FBLc03-108 cells/mL and L. casei FBLc03-106 cells/mL had the highest values and not significantly different. The results of sensory evaluation showed that most treatments were not statistically different. However, treatment using L. casei FBLc01-108 cells/mL had the lowest sensory score (14.4 ± 0.71/20) while the treatment using L. casei FBLc03-106 cells/mL had the highest sensory score (18.1 ± 1.16/20). Therefore, L. casei FBLc03-106 cells/mL was chosen for further experiments.

3.2 The effect of corn milk/cow milk ratio and sugar concentration

As shown in Table 2, with the same ratio between corn and cow milk, the treatments had a higher amount of sugar added, TA value was low, and pH was high. Besides, if the same amount of adding sugar and different corn/cow milk ratios, pH value had the inverse proportion with the amount of adding corn milk, when the content of corn milk was low, the pH value was high and TA value was low.

According to the appropriate conditions for pH value and TA of yogurt products (pH of 4.1-4.6 and TA of 0.7-0.9%), 10 treatments including 1:9-4%, 2:8-4%, 2:8-8%, 2:8-12%, 3:7-4%, 3:7-8%, 4:6-4%, 4:6-8%, 4:6-12%, and 5:5-16% had pH values around 4.21-4.60 and TA values were in the range of 0.7-0.9%. Of which, the treatment of 2:8-12% had the highest sensory score (19.1 ± 1.01/20) so this treatment was chosen for further experiments..

Table 2. Fermentation of sweet corn milk yogurt with different ratios of corn/cow milk and sugar concentrations.

Sweet corn milk/cow milk	Adding sugar (%)	pH	Total acidity (%)	Sensory score
5:5	4	3.70 ± 0.04^{a}	0.97 ± 0.02^{a}	8.9 ± 0.61^{ef}
5:5	8	4.86 ± 0.11^{jk}	0.63 ± 0.00^{i}	12.1 ± 1.07^{de}
5:5	12	4.65 ± 0.09^{fghi}	0.68 ± 0.00^{fg}	15.8 ± 0.79^{abc}
5:5	16	4.32 ± 0.02^{bcd}	0.79 ± 0.01^{bc}	15.3 ± 0.57^{bcd}
4:6	4	4.23 ± 0.11^{bc}	0.81 ± 0.02^{b}	8.3 ± 0.11f
4:6	8	4.52 ± 0.04^{fg}	0.72 ± 0.01^{ef}	13.7 ± 0.35^{de}
4:6	12	4.46 ± 0.00^{def}	0.74 ± 0.01^{de}	15.4 ± 0.83^{bcd}
4:6	16	4.87 ± 0.03^{k}	0.64 ± 0.00^{hi}	16.3 ± 2.04^{abc}
3:7	4	4.29 ± 0.08^{bc}	0.80 ± 0.01^{bc}	10.2 ± 0.54^{ef}
3:7	8	4.21 ± 0.13^{b}	0.82 ± 0.01^{b}	16.2 ± 0.61^{abc}

3:7	12	4.72 ± 0.18^{ij}	0.67 ± 0.00^{gh}	15.8 ± 1.33^{abc}
3:7	16	4.87 ± 0.34^{k}	0.64 ± 0.01^{h}	15.9 ± 0.74^{abc}
2:8	4	4.37 ± 0.16^{cde}	0.77 ± 0.03^{cd}	9.1 ± 0.23^{ef}
2:8	8	4.48 ± 0.07^{ef}	0.74 ± 0.04^{de}	15.7 ± 0.67^{bc}
2:8	12	4.55 ± 0.14^{gh}	0.73 ± 0.03^{de}	19.1 ± 1.01^{a}
2:8	16	5.33 ± 0.12^{l}	0.54 ± 0.00^{j}	16.9 ± 0.79^{abc}
1:9	4	4.60 ± 0.03^{fghi}	0.70 ± 0.01^{efg}	9.9 ± 0.30^{ef}
1:9	8	4.67 ± 0.20^{hi}	0.68 ± 0.02^{g}	17.4 ± 0.92^{ab}
1:9	12	4.96 ± 0.06^{k}	0.62 ± 0.02^{i}	19.1 ± 1.05^{a}
1:9	16	5.50 ± 0.17^{m}	0.50 ± 0.01^{j}	18.3 ± 0.94^{ab}
Coefficient of variation (%)		7.62	9.12	13.65

Note: Values in the table were the average values of triplications. Difference values of statistics only had meaning in the column; the average values with the same letter were not significant at 95% probability.

3.3 The effect of fermentation temperature and time

After incubation in 15 h, all treatments reached the required values of pH and TA (Table 3). While the pH values of treaments at 37°C and 40°C were not statistically different, the pH value at 30°C was higher and had a statistical difference. At the same temperature, if products were incubated in the longer fermentation time, it had lower pH. When the starter was added to the mixture, it needs time to adapt new environment and their enzyme system to some extent to reach the maximum growth rate [4]. In the early periods, pH values were high (6.37 at 30°C, 5.65 at 37°C, and 5.56 at 40°C) after 6 h and the amount of acid lactic was low (0.36 ± 0.01% at 30°C, 0.48 ± 0.03% at 37°C, and 0.49 ± 0.03% at 40°C). After 12 h, at 37°C and 40°C, treatments got required pH and TA values for sweet corn milk yogurt. In the same range of fermentation time, when treatments were incubated at higher temperature it had lower pH. At the end of fermentation period, all values had good results for the final product. The highest sensory scores belonged to treatments at 37°C and 40°C with 12 h and 15 h of fermentation. These 4 treatments had no significantly different. However, to save time and energy, treatment of 37°C and 12 h was the most suitable.

Table 3. Fermentation of sweet corn milk yogurt at different temperatures and time.

Temperature (°C)	**Time (h)**	**pH**	**Total acidity (%)**	**Sensory score**
30	6	6.37 ± 0.22^{g}	0.36 ± 0.01^{g}	7.8 ± 0.13^{f}
	9	5.83 ± 0.13^{h}	0.45 ± 0.01^{f}	9.1 ± 0.34^{ef}
	12	4.82 ± 0.21^{c}	0.66 ± 0.03^{b}	10.8 ± 0.24^{de}
	15	4.65 ± 0.09^{b}	0.71 ± 0.02^{b}	11.8 ± 0.45^{cd}
37	6	5.65 ± 0.12^{e}	0.48 ± 0.03^{e}	8.1 ± 0.31^{f}
	9	5.30 ± 0.14^{d}	0.54 ± 0.00^{d}	12.9 ± 0.23^{bc}

	12	4.61 ± 0.07^{b}	0.69 ± 0.01^{b}	16.1 ± 0.32^{a}
	15	4.49 ± 0.11^{a}	0.73 ± 0.02^{a}	16.4 ± 0.21^{a}
40	6	5.56 ± 0.07^{e}	0.49 ± 0.03^{e}	7.7 ± 0.17^{f}
	9	5.25 ± 0.16^{d}	0.56 ± 0.04^{d}	13.6 ± 0.31^{b}
	12	4.59 ± 0.19^{b}	0.70 ± 0.02^{b}	16.2 ± 0.45^{a}
	15	4.45 ± 0.05	0.75 ± 0.02^{a}	16.9 ± 0.42^{a}
Coefficient of variation (%)		8.12	9.61	11.04

Note: Values in the table were the average values of triplications. Difference values of statistics only had meaning in the column; the average values with the same letter were not significant at 95% probability.

3.4 The effect of the storage condition in sweet corn milk yogurt

pH values of sweet corn milk yogurt stored at 0°C after 1 month had not significantly change with a slight decrease, from 4.57 ± 0.17 (1st week) to 4.51 ± 0.12 (4th week) but still in the suitable range of normal yogurt (Figure 2A). The TA values also had no statistical different change during 4 weeks of storage. The final TA value was 0.73 ± 0.03% and the cell number was at 9.86 ± 0.61 log cells/mL (Figure 3). Although LAB almost stop all their growth at 0°C [4], there is little change in pH, TA and cell density values during storage.

Normally, when temperature is around 5oC or lower, the latic acid fementation was not continued to produce [4]. However, if the yogurt was stored at 5°C in a long time, the amount of lactic acid would increase gradually day by day. At the second week of storage, the pH and TA values were slightly changed to 4.11 ± 0.13 and 0.85 ± 0.03% (Figure 2B). The overall sensory characteristic of corn milk yogurt at second week of storage was not clearly change but the product become to bad smell and taste at the end of the third week. The TA value reached 1.21 ± 0.04% and pH value dramatically decreased to 2.89 ± 0.11. Cell number was 10.12 ± 0.73 log cells/mL (Figure 3) on the last day of 3 weeks of storage. The pH and acidity of corn milk yogurt produced by L. casei TISTR390 were 3.87 and 1.12%, respectively, after storage at 5°C for 15 days [27]. Supavititpatana et al. [28] also reported that the shelf-lives of corn milk and cow milk yogurts were 14 days at 5°C.

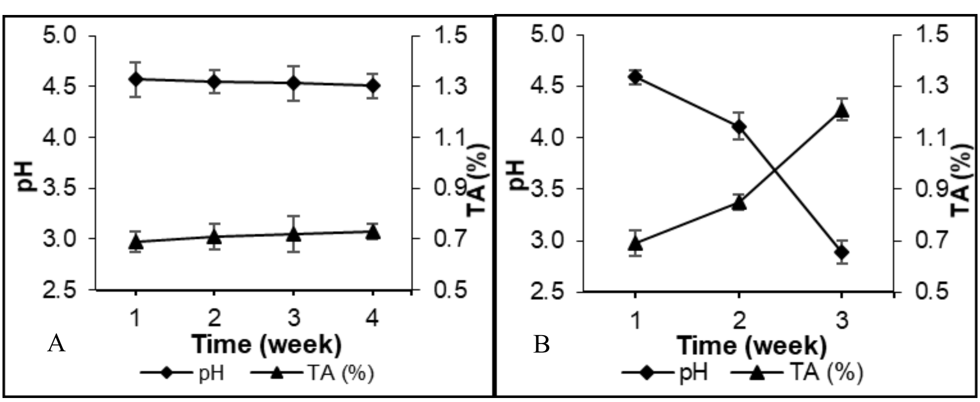

Fig. 2. The pH and TA values during storage of sweet corn milk yogurt at 0°C (A) and 5°C (B)

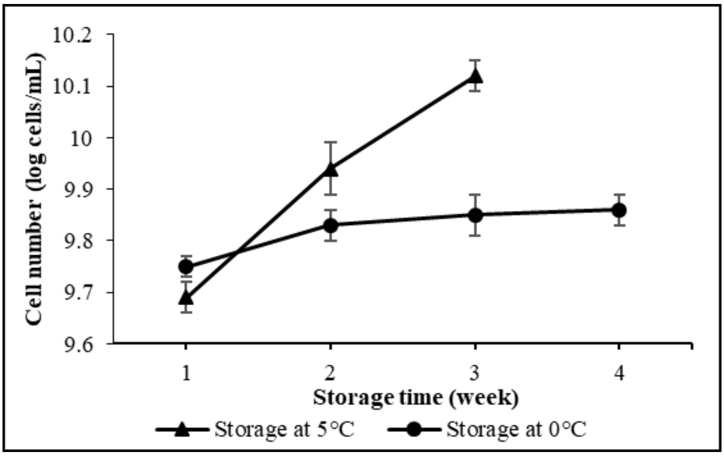

Fig. 3. Change of *L. casei* cell density in sweet corn milk yogurt storage at 0°C and 5°C

4 Conclusion

L. casei FBLc03 has the good performance in lactic acid fermentation with initial cell density at 106 cells/mL. The proportion of corn milk and cow milk was 2:8 with 12% of adding sugar, and the suitable fermentation temperature was 37°C for 12 h. Sweet corn milk yogurt remained acceptable taste and texture until a month at 0°C and 2 weeks at 5°C..

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