International Food Research Journal 23(4): 1733-1738 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Production of corn milk yogurt supplemented with probiotics

Trikoomdun, W. and *Leenanon, B.

Department of Food Technology, Faculty of Technology, Khon Kaen University, Khon Kaen, 40002, Thailand

Article history

Received: 5 August 2015
Received in revised form: 18 October 2015
Accepted: 8 November 2015

Keywords

Corn milk
Yogurt
Stabilizer
Probiotics

Abstract

Probiotics are live microorganisms which are health benefits via balancing the microorganisms in the intestinal system. Corn milk samples prepared from sweet corn and water at three different ratios as 1:1, 1:2 and 1:3 were used to produce corn milk yogurt and it was found that corn milk yogurt made from sweet corn and water at 1:2 ratio gave the highest overall liking score but it was not significantly different from the one made from 1:3 ratio. Then, two types of stabilizers including gelatin and carboxymethylcellulose (CMC) at the concentration of 0, 0.2, 0.4, and 0.6% were added to yogurt and the result showed that gelatin at 0.2% was the suitable one which contributed the highest overall liking score. Moreover, different levels of yogurt starter at 2, 3, 4 and 5% were determined for corn milk yogurt production and it revealed that 2% yogurt starter was the minimum amount which contributed the lowest percentages of syneresis with insignificant differences in overall liking scores. Growth curves of *Lactobacillus acidophilus* TISTR1338 and *Lactobacillus casei* TISTR390 were also investigated and it was shown that both strains would grow into the stationary phase after 28 hrs of incubation at 37°C with the numbers of 9 log cfu/ml. Finally, both probiotic strains were supplemented in corn milk yogurt and determined for their survival during storage at 5°C for 15 days. It was shown that *L. casei* TISTR390 could survive significantly better than *L. acidophilus* TISTR1338 with the numbers of 9.11 and 8.76 log cfu/g respectively which were higher than the minimum therapeutic dose (6 log cfu/g).

Introduction

Nowadays functional foods have an important role in daily life because people are more concerned on their health and benefits from consumed foods (Saxelin, 2008). The changes in consuming behavior towards healthy foods including main course, dessert or supplemented foods are noticed these days. Some of interesting methods is applying probiotics to many kinds of foods such as ice cream, yogurt, fruit juice or salad dressing (Franz *et al*., 2014). Probiotics are viable microorganisms that give benefits and healthy from consumed foods. Probiotic foods have to contain viable probiotic bacteria at least 10^6 cells/ml (Kurmann and Rasic, 1991). Since the consumers these days are aware of the health related issues; therefore, the production of yoghurt based products has focused mainly on the supplement of probiotic microorganisms into the products. The probiotics are beneficial microbes in the human gastrointestinal tract which contribute several therapeutic effects such as reducing serum cholesterol level, treating inflammatory bowel disease, preventing gastrointestinal problems, increasing mineral bioavailability and its immunological effects (Lorea-Baroja *et al*., 2007; Anukam *et al*., 2008).

Yogurt, one of the fermented milk products, is a functional food which is popular and acceptable from consumers for several years and amounts of consumers are growing by 30% because yogurt is a high nutritious food containing α-lactalbumin, β-lactoglobulin, vitamin A, calcium and phosphorus (Fuller, 1989). Yogurt is usually made from cow milk with the action of two homofermentative lactic acid bacteria, *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and had a final pH value of 3.8-4.6 (Lourens-Hattingh and Viljoen, 2001).

However, some researchers have made yogurt from different types of raw materials other than cow milk including goat milk, buffalo milk, soy milk, and peanut milk (Granata and Morr, 1996). Thus, yogurt making from corn milk would also be challenging to be investigated since corn milk has been processed from sweet corn and it has a nice color, aroma and appearance, along with its sweet taste, either pasteurized or sterilized (Pulham, 1997; Supavititpatana *et al*., 2008). Corn milk is well accepted among health conscious consumers, since it has nutritional values over other types of vegetable

*Corresponding author.
Email: *boris394135@gmail.com*

drink as it is high in vitamin content, including vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin C and niacin and it is also low in saturated fat and cholesterol (USDA, 2004).

Texture is an important characteristic of yogurt quality and the addition of a stabilizer such as gelatin or carboxymethylcellulose (CMC) may provide a great stability and suitable texture preventing from syneresis via binding with water and may also interact with protein in the food system (Tamime and Robinson, 1999; Duboc and Mollet, 2001; Kumar and Mishra, 2004). This research was aimed to process corn milk into yogurt with the suitable type and level of stabilizer and also add up with probiotic bacteria. Thus, the probiotic corn milk yogurt achieved would be an interesting functional food which contributes both nutritional values and probiotics to consumers.

Materials and Methods

Chemical composition of sweet corn

The sweet corn *(Zea mays saccharata)* was purchased from Suwan Farm, Pakchong, Nakhon Ratchasima province, Thailand. They were harvested at milking stage about 19-21 days after silking (Pulham, 1997). The whole corn was husked, silk removed, and finally cleaned. The corn kernels were separated from its cob and determined for protein, carbohydrate, and ash content (AOAC, 2000).

The suitable corn kernels and water ratio for preparing corn milk yoghurt

Corn milk were prepared from corn kernels and water at the ratio of 1:1, 1:2, and 1:3 (w/w), then heated at 95°C for 10 minutes and cooled down to room temperature. After that, the mixtures were blended for 3 minutes and filtered through nylon to achieve corn milk. For yoghurt preparation, corn milk with three different ratios were added with skim milk to achieve 16% total solids and then heated at 85°C for 30 minutes, cooled down to 43-45oC then 2%(v/v) commercial yogurt starter (Yo-Flex®:YC-380) including *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was added. After that, they were incubated at 43°C and sampled every three hours for determination of pH (AOAC, 2000) and %acidity (AOAC, 2000). Also, % syneresis which was the extent of whey separation (free whey) collected on the top or around the sides of the gel and expressed as a percentage of the total milk weight (Lucey *et al*., 1998; Lucey, 2001) and sensory evaluation (color, odor, sourness, flavor and overall liking) of corn milk yoghurt using 9-point Hedonic scale by 30 untrained panelists were conducted.

Statistical analysis was performed using SPSS statistical software program version 17. The chemical and physical properties of yogurt samples were analyzed by analysis of variance using Completely Randomized Design whereas the sensory evaluations of yogurt samples were analyzed by analysis of variance using Randomized Complete Block Design. If the F-value was significant, the Duncan's New Multiple Range Test was used to determine differences between the treatment means and P values of ≤0.05 were regarded as significant.

The suitable type and level of stabilizers for making corn milk yogurt

Yogurt was prepared from corn milk with the suitable ratio of corn to water. Gelatin or carboxymethylcellulose (CMC) was also added at 0 (control sample), 0.2, 0.4, and 0.6%. Then, the mixtures were heated at 85°C for 30 minutes, cooled down to 43-45°C and 2% (v/v) commercial yogurt starter (Yo-Flex®:YC-380) including *L. bulgaricus* and *S. thermophilus* was added. After that, they were incubated at 43°C and sampled every three hours for determination of pH (AOAC, 2000) and %acidity (AOAC, 2000). Also, %syneresis (Lucey, 2001) and sensory evaluation (color, odor, sourness, flavor and overall liking) using 9-point Hedonic scale by 30 untrained panelists were conducted.

Statistical analysis was performed using SPSS statistical software program version 17. The chemical and physical properties of yogurt samples were analyzed by analysis of variance using 2×4 Asymmetric Factorial Experiment in Completely Randomized Design whereas the sensory evaluations of yogurt samples were analyzed by analysis of variance using 2×4 Asymmetric Factorial Experiment in Randomized Complete Block design. If the F-value was significant, the Duncan's New Multiple Range Test was used to determine differences among the treatment combination means and P values of ≤0.05 were regarded as significant.

The suitable level of yogurt starter for corn milk yogurt fermentation

Yogurt was prepared from corn milk with suitable ratio of corn to water and also added with suitable type and level of stabilizer. Then, the mixtures were heated at 85°C for 30 minutes, cooled down to 43-45°C. The commercial yogurt starter (Yo-Flex®: YC-380) including *L. bulgaricus* and *S. thermophilus* was added at 2, 3, 4, or 5% (v/v). After that, they were incubated at 43°C and sampled every three hours for determination of pH (AOAC, 2000) and %acidity (AOAC, 2000). Also, %syneresis (Lucey, 2001) and sensory evaluation (color, odor, sourness, flavor and overall liking) using 9-point Hedonic scale by 30 untrained panelists were conducted.

Statistical analysis was performed using SPSS statistical software program version 17. The chemical and physical properties of yogurt samples were analyzed by analysis of variance using Completely Randomized Design whereas the sensory evaluations of yogurt samples were analyzed by analysis of variance using Randomized Complete Block Design. If the F-value was significant, the Duncan's New Multiple Range Test was used to determine differences between the treatment means and P values of ≤0.05 were regarded as significant.

Growth curves determinations of probiotic bacteria

Probiotic bacteria including *L. acidophilus* TISTR1338 and *L. casei* TISTR390 were transferred to MRS broths (aseptic technique) and incubated at 37°C for 48 hours. Then they were subcultured in new MRS broth media 2-3 times for culture activation. The cultures were also stabbed in MRS agar butt, incubated at 37°C for 48 hours and subsequently kept at low temperature (4°C) for using as working stock cultures.

The activated cultures were inoculated into 100 ml MRS broth and incubated at 37°C for 48 hours. Then, they were plated with MRS agar and incubated at 37°C for 48 hours. The initial numbers of probiotics were enumerated as cfu/ml. After that, they were diluted with 99 ml, 0.1% peptone water and 99 ml, MRS broth to achieve 10^2 cfu/ml and incubated at 37°C for 48 hours. During incubation, they were sampled and determined for absorbance at 600 nm wavelength (OD_{600}) along with total plate count (log cfu/ml) in MRS Agar. Finally, growth curves were constructed with total plate count (log cfu/ml) and OD_{600} as y-axis and time (hours) as x-axis.

Survival of L. acidophilus *TISTR1338 and* L. casei *TISTR390 in corn milk yogurt*

Probiotic bacteria including *L. acidophilus* TISTR1338 and *L. casei* TISTR390 were transferred to MRS broth (aseptic technique) and incubated at 37°C until they grew into the stationary phase. Probiotic bacteria i.e. *L. acidophilus* TISTR1338 and *L. casei* TISTR390 growing into the stationary phase with the numbers of 10^8 cfu/ml were separately added to corn milk yogurt after fermentation and then kept at 5ºC for 15 days. During storage, they were sampled every three days for determination of pH (AOAC, 2000), %acidity (AOAC, 2000) and counts of *L. acidophilus* TISTR1338 and *L. casei* TISTR390 using selective media as MRS bile agar and Vancomycin agar respectively.

Statistical analysis was performed using SPSS statistical software program version 17. The chemical properties and probiotic counts of yogurt samples were analyzed by analysis of variance using Completely Randomized Design. If the F-value was significant, the Duncan's New Multiple Range Test was used to determine differences between the treatment means and P values of ≤0.05 were regarded as significant.

Results and Discussion

Chemical composition of sweet corn

The chemical composition of sweet corn (Insri2) particularly protein, carbohydrate, and ash were 13.31, 69.06, and 2.94% respectively which were similar to the gross chemical composition of sweet corn compiled by Lunven (1992) as 12.9% protein, 69.3% carbohydrate and 1.5% ash.

The suitable ratio of corn kernels to water for preparing corn milk yoghurt

According to Figure 1, it was shown that during incubation at 42°C for 12 hours, pH tended to decrease whereas % acidity tended to increase particularly from 2 to 6 hours. Also, after 12 hours incubation, pHs of corn milk yogurt at the ratios of corn to water as 1:1, 1:2 and 1:3 were significantly different as 4.03, 3.81, and 3.76 respectively while % acidity of those were not significantly different as 1.77, 1.87, and 1.76 respectively. Tamine and Robinson (1999) had found that good quality yogurt had a final pH and %lactic acid as 3.8-4.2 and 0.9-1.2 respectively.

After incubated at 42°C for 12 hours, % syneresis of corn milk yogurt formula 3 (1:3 ratio) gave highest value followed by formula 2 (1:2 ratio) and 1 (1:1 ratio) as 7.81, 3.72 and 0.55% respectively (P≤0.05).

It was found that color and odor scores of corn milk yogurt with all formula were not significantly different (P>0.05) while texture and sourness scores of corn milk yogurt with formula 1 and 3 were significantly different (P≤0.05). Interestingly, the overall liking score of corn milk yogurt with formula 2 contributed the highest score (P≤0.05) but was not significantly different from the one of formula 3 (P>0.05). Thus, corn milk yogurt with formula 2 was considered appropriate for further study since more corn kernels were used.

Effects of types and levels of stabilizers on quality of corn milk yogurt

From Table 1, it was shown that there was an interaction between levels and types of stabilizer

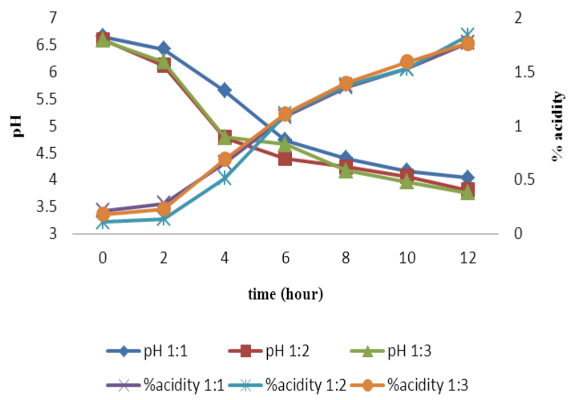

Figure 1. pH and % acidity changes of corn milk yogurt incubated at 42°C for 12 hours

Table 1. % Syneresis of corn milk yogurt added with different types and levels of stabilizers after incubated at 42°C for 6 hours

Types	Levels (%)			
	0	0.2	0.4	0.6
CMC	2.91^{a}±0.17	0.75^{d}±0.10	0.56^{de}±0.07	0.46^{e}±0.04
gelatin	2.91^{a}±0.17	1.43^{b}±0.30	0.99^{c}±0.11	0.75^{d}±0.13

Table 2. pH, %acidity and % syneresis of corn milk yogurt using different yogurt starter levels after incubated at 42°C for 6 hours

%yogurt starter	pH	% acidity	% syneresis
2	3.82^{a}±0.07	0.84^{a}±0.10	1.16^{c}±0.01
3	3.81^{a}±0.06	0.82^{a}±0.08	1.50^{b}±0.12
4	3.82^{a}±0.01	0.83^{a}±0.05	2.58^{a}±0.08
5	3.73^{b}±0.09	0.93^{a}±0.12	2.65^{a}±0.02

(P≤0.05) as % syneresis decreased with increasing level of stabilizer and also gelatin contributed % syneresis higher than that of CMC (P≤0.05).

Supavititpatana *et al.* (2008) found that gelatin added in corn milk yogurt had an effect on %syneresis. Using high level of gelatin would reduce syneresis because water phase reduction by gelatin in yogurt structure reduced rapidity of syneresis (Modler and Kalab, 1983; Keogh and O'Kennedy, 1998; Fiszman *et al*., 1999).

It was shown that odor and sourness scores of corn milk yogurt were not significantly different (P>0.05) when using CMC and gelatin as stabilizer whereas color and overall liking scores of corn milk yogurt using gelatin contributed higher score than CMC (P≤0.05).

Also, it was shown that color, odor and sourness of corn milk yogurt added with different levels of stabilizer were not significantly different (P>0.05) whereas the overall liking scores of corn milk yogurt using stabilizer at 0.2, 0.4, and 0.6% were not significantly different (P>0.05) but they were all significantly higher than 0% (control) (P≤0.05). Moreover, very high level of stabilizer affected on the palatability as it would be reduced in the term of gelling characteristic (Lucey, 2004). Therefore, 0.2% gelatin was selected as stabilizer since it contributed the highest score with the lowest amount of stabilizer.

Effects of yogurt starter levels on quality of corn milk yogurt

From Table 2, it was shown that pH of corn milk yogurt using yogurt starter at 2, 3, and 4% were 3.82, 3.81 and 3.82 respectively which were significantly higher than the one using yogurt starter at 5% that contributed pH as 3.73 (P≤0.05). In addition, % acidity of corn milk yogurt using yogurt starter at 2, 3, 4 and 5% were 0.84, 0.82, 0.83, and 0.93 respectively which were not significantly different (P>0.05). Surprisingly, 2% yogurt starter contributed the lowest of %syneresis as 1.16 compared to the ones using 3, 4 and 5% yogurt starter with % syneresis as 1.50, 2.58 and 2.65 respectively (P≤0.05).

It was shown that color, odor, texture, sourness and overall liking scores of corn milk yogurt using 2, 3, 4 and 5% yogurt starter levels were not significantly different with the scores of overall liking as 5.27, 5.33, 5.57, and 5.13 respectively (P>0.05). Therefore, 2% yogurt starter level was selected for further study since it was a lowest level.

Olson *et al.* (2008) studied the effects of adding *L. acidophilus* in yogurt on pH, survival, syneresis, color and panel acceptance and it was found that whey released from yogurt when *L. acidophilus* was added with different levels affected on different syneresis (P≤0.0001). The syneresis was increased when using higher level of *L. acidophilus* from 0.0239 to 2.33 grams of yogurt and level of yogurt starter was also an important factor for final level of microorganism. Anon (1994) founded that using over amount of *L. bulgaricus* and *S. thermophilus* would decrease the final level of *L. acidophilus* and *B. bifidum*.

Growth curves of L. acidophilus *TISTR1338 and* L. casei *TISTR390*

Growth curves of *L. acidophilus* TISTR1338 and *L. casei* TISTR390 for using as probiotic in corn milk yogurt were shown in Figure 2. Figure 2 showed the

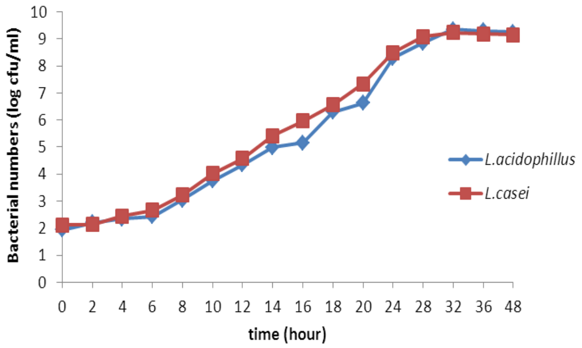

Figure 2. Bacterial numbers of *Lactobacillus acidophilus* TISTR1338 and *Lactobacillus casei* TISTR390 in MRS broth incubated at 37°C for 48 hours

number changes of *L. acidophilus* TISTR 1338 and *L. casei* TISTR390 from hour 0 to hour 4 (lag phase). Both of them then grew into log phase after 6 hours until hour 28 and started growing into stationary phase with a number of 9 log cfu/ml. *L. acidophilus* TISTR1338 and *L. casei* TISTR390 growing in the stationary phase had been applied as probiotics in corn milk yogurt.

Application of L. acidophilus *TISTR1338 and* L. casei *TISTR390 as probiotics in corn milk yogurt*

According to Table 3, pH of corn milk yogurt with added probiotics, *L. acidophilus* TISTR1338 and *L. casei* TISTR390, were not significantly different as 3.75 and 3.87 respectively while %acidity of corn milk yogurt with *L. casei* TISTR390 was significantly higher than the one of *L. acidophilus* TISTR1338 as 1.17 and 1.12 respectively ($P \leq 0.05$) after storage at 5°C for 15 days.

For viable probiotic cells, it was found that *L. casei* TISTR390 could survive significantly better than *L. acidophilus* TISTR1338 with the numbers of 9.11 and 8.76 log cfu/g respectively ($P \leq 0.05$). In addition, viable cells of both probiotic strains were higher than the minimum recommended therapeutic level of 6 log cfu/ml after 15 days storage at 5°C (Kurmann and Rasic, 1991). Also, McCann *et al.* (1996) reported that *L. casei* was added in yogurt as probiotic bacteria because it was more stable than *L. acidophilus* or bifidobacteria.

Conclusion

Corn milk prepared from sweet corn and water at a ratio of 1:2 along with 0.2% gelatin as stabilizer was used to produce corn milk yogurt which contributed the highest overall liking score. Moreover, 2% yogurt starter was the suitable level which gave the lowest percentages of syneresis with insignificant

Table 3. pH, %acidity and viable probiotic cells of corn milk yogurt stored at 5°C for 15 days

Probiotics	pH	% acidity	Viable probiotic cells (log cfu/g)
L. casei TISTR390	3.75[a]±0.03	1.17[a]±0.01	9.11[a]±0.09
L. acidophilus TISTR 1338	3.87[a]±0.04	1.12[b]±0.01	8.76[b]±0.04

differences in overall liking scores. Growth curves of *L. acidophilus* TISTR1338 and *L. casei* TISTR390 showed that both strains would grow into the stationary phase after 28 hours of incubation at 37°C with the numbers of 9 log cfu/ml. Finally, it was shown that *L. casei* TISTR390 could survive significantly better than *L. acidophilus* TISTR1338 with the numbers of 9.11 and 8.76 log cfu/g respectively which were higher than the minimum therapeutic dose (6 log cfu/g). Hence, probiotic corn milk yogurt produced could be considered as one of the potential probiotic foods which contribute benefits to consumers.

Acknowledgements

This research was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Food and Functional Food Research Cluster of Khon Kaen University.

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