

Effect of Capsule Storage on Viability of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in Yogurt Powder

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Abstract—Yogurt capsule was made by mixing 14% w/v of reconstitution of skim milk with 2% FOS. The mixture was fermented by commercial yogurt starter comprising *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These yogurts were made as yogurt powder by freeze-dried. Yogurt powder was put into capsule then stored for 28 days at 4°C. 8ml of commercial yogurt was found to be the most suitable inoculum size in yogurt production. After freeze-dried, the viability of *L. bulgaricus* and *S. thermophilus* reduced from 10^9 to 10^7 cfu/g. The presence of sucrose cannot help to protect cell from ice crystal formation in freeze-dried process, high (20%) sucrose reduced *L. bulgaricus* and *S. thermophilus* growth during fermentation of yogurt. The addition of FOS had reduced slowly the viability of both *L. bulgaricus* and *S. thermophilus* similar to control (without FOS) during 28 days of capsule storage. The viable cell exhibited satisfactory viability level in capsule storage (6.7×10^6 cfu/g) during 21 days at 4°C.

Keywords—Yogurt capsule, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, freeze-drying, sucrose.

I. INTRODUCTION

YOGURT made from fermentation of milk by probiotic bacteria, milk ferments and coagulates to thicken the milk to a creamy texture. *Lactobacillus acidophilus* and *Streptococcus thermophilus* are probiotic bacterial are usually used as culture, although some manufacturers use *L. bulgaricus* rather than *L. acidophilus*. Yogurt is popular and known to be as health food contains protein, calcium, potassium, iodine and vitamins B. It may also help to prevent osteoporosis, colon cancer, inflammatory bowel disease, reduce lactose intolerance, stimulate immune system, inhibition of pathogenic bacteria, cholesterol absorption [1], [2] and improving the intestinal microbial balance [5]. However, amount of probiotic is essential that there is a minimum of one million viable probiotic organisms per gram of product [3].

Today yogurt is popular in women. It can help improve the health and quality of skin. However, many people don't like taste of yogurt but need to eating for healthy. Yogurt powder contains in capsule is interesting due to easy to eat like a vitamin and supplement capsules. Freeze-drying yogurt is a process that preserves yogurt and maintains a sufficient quantity of viable probiotics. Previous research has found that certain strain of probiotics are better able to survive the freeze-

drying process due to the frozen water is removed by sublimation thus reduce damage to structure [7].

Cryoprotectant is a substance used to protect cell from freezing damage. In addition to being water soluble, good cryoprotectants are effective at depressing the melting point of water, do not precipitate or form eutectics or hydrates, and are relatively non-toxic to cells at high concentration. All cryoprotectants form hydrogen bonds with water. In cryopreservation by freezing or vitrification, more than half of the water inside cells is ultimately replaced by cryoprotectant molecules. Cryoprotection can be regarded as a process of replacing water molecules with other molecules that cannot freeze [6], common cryoprotectant such as dimethyl sulfoxide (DMSO), ethylene, glycol, glycerol, 2-methyl-2,4-pentanediol (MPD), propylene glycol and sucrose. Sucrose are also used to preserve foods, they do not pose any toxicity concerns. Yogurt production, sucrose are added into the yogurt for increase sweetness flavor. Development of yogurt product with new product has potential health benefits help to increasing sale. Capsules are used to enclose medicines and can be taken orally. Hard-shelled capsules are typically made from gelatin and contain dry, powdered ingredients or miniature pellets. The objective of this present research was to study the survival of *L. bulgaricus* and *S. thermophilus* in yogurt powder made from freeze-dried process and study the effect of storage of yogurt powder in capsule on viability of *L. bulgaricus* and *S. thermophilus*.

II. MATERIALS AND METHODS

A. Probiotic Bacteria

Probiotic bacterial were obtained from commercial yogurt (dutchie bio, Dutch Mill Co., Ltd, Thailand) including two strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*

B. Effect of Inoculum Size on Viability of Probiotic

Yogurts were prepared by mixing 100 ml of heat treat (85°C for 30 min) and cooled 14% w/v reconstitution of skim milk (RSM) with 2ml, 4ml, 6ml, 8ml and 10ml starter commercial yogurt followed by incubated at 42°C for 3-6 h. until pH reached 4.5. *L. bulgaricus* and *S. thermophilus* were enumerated before and after fermentation.

C. Effect of Sucrose on Survival of *L. bulgaricus* and *S. thermophilus* in Freeze-Dried Yogurt

Sucrose is chosen as cryoprotectant for protect from freeze-

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dried process, 8 ml of commercial yogurt starter and 2%, 4%, 6%, 8% and 10% sucrose were added to the heat treat (85°C for 30 min.) and cooled 14% w/v RSM, followed by incubated at 42°C for 4-6 h until pH reached 4.5. Yogurts were then placed in the refrigerated at -20°C for 24 h and dried in vacuum at -75°C. *L. bulgaricus* and *S. thermophilus* were enumerated before and after freeze-dried.

D. Preparation of Yogurt Capsule

Yogurt was made powder by freeze-dried process, 8ml commercial yogurt starter and 2% FOS were added to the heat treat (85°C for 30 min) and cooled 14% w/v RSM. 1ml of each mixture was placed into small tubes followed by incubated at 42°C for 3-6 h. until pH reached 4.5. Yogurts were then placed in the refrigerated at -20°C for 24 h. and dried in vacuum at -75°C. 500 mg of yogurt powder was put into capsule and packed in aluminium bag. The bags were stored at 4°C in the refrigerated for 28 days. *L. bulgaricus* and *S. thermophilus* were enumerated before, after freeze-dried and during 0, 7, 14, 21, 28 days of capsule storage. Statistical significance of data analyses were performed using Independent Sample Test, ($p < 0.05$).

E. Enumeration of Probiotic Bacteria

L. bulgaricus and *S. thermophilus* were enumerated by mixing 500 mg yogurt powder with 9.5 ml 0.85% sterile NaCl and serial dilutions were prepared by using 0.85% NaCl as the diluents. 0.1 ml diluted yogurt was then transferred in the Lactobacillus bulgaricus agar (for *L. bulgaricus*) and Streptococcus thermophilus agar (for *S. thermophilus*) and was spreaded on surface agar using a sterile spreader. The plates were incubated anaerobically in candle jars at 42°C for 48 h. Viable microbial count was calculated as follows.

$$\frac{\text{cfu/ml} = \text{number of colonies formed} \times \text{dilution factor}}{0.1 \text{ mL of sample}}$$

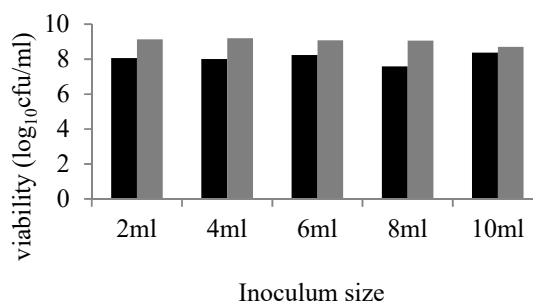
where cfu is the colony forming unit.

III. RESULTS AND DISCUSSION

A. Effect of Inoculum Size on Viability of Probiotic

Effect of inoculum size on *L. bulgaricus* and *S. thermophilus* counts were shown in Fig. 1. Before fermentation of milk by commercial yogurt starter at 2 ml, 4ml, 6ml, 8ml and 10 ml, *L. bulgaricus* and *S. thermophilus* counts were about 10^8 and 10^7 cfu/ml, respectively. Viable cell count had increased after fermentation in all inoculum size, especially 8ml of inoculum size increased two-log both *L. bulgaricus* and *S. thermophilus*. At 10 ml of inoculum size, *L. bulgaricus* and *S. thermophilus* counts small increased may have resulted from competition of microbial for using nutrients in milk. According to the result, 8 ml of inoculum size was used as yogurt starter for yogurt capsule production.

1. *L. bulgaricus*



2. *S. thermophilus*

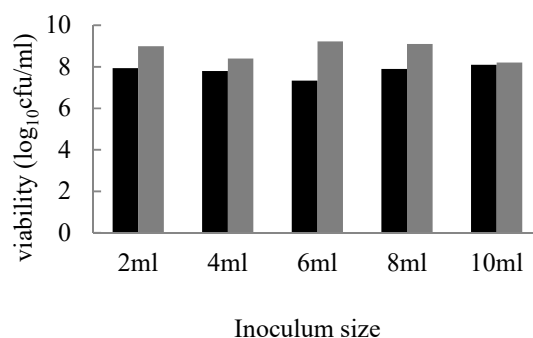


Fig. 1 Effect of inoculum size on viability of *L. bulgaricus* (A) and *S. thermophilus* (B) in yogurt before fermentation () and after fermentation ()

B. Effect of Sucrose on Survival of *L. bulgaricus* and *S. thermophilus* in Yogurt Freeze-Dried

The presence of sucrose (5%, 10%, 15% and 20%) resulted in lower *L. bulgaricus* and *S. thermophilus* counts during yogurt fermentation compared with control (10^9 cfu/g) especially, 20% sucrose *L. bulgaricus* and *S. thermophilus* counts was about 10^7 cfu/g after fermentation (TABLE I). The reduction of *L. bulgaricus* and *S. thermophilus* growth which occurred from osmosis effected. After freeze-dried, the viable cell counts reduced in all yogurts (Fig. 2) may have resulted from freeze injury to the viable cell. The presence 20% sucrose did exhibit considerable resistance to the freezing process, demonstrating an about 80% survival rate after freezing compared with about 70% for another degree of sugar. The toxicity of cryoprotectants administered at near-freezing temperatures is a different kind of toxicity than the toxicity experienced by living things at warm temperature [6]. It can be concluded that the addition of sucrose did not protect cell from ice crystal formation, may result in damage of bacterial cells and reduced viability.

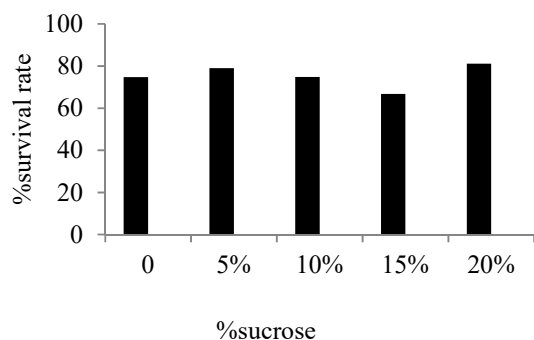
TABLE I
COUNTS OF *L. BULGARICUS* AND *S. THERMOPHILUS* IN YOGURT BEFORE AND AFTER FREEZE-DRY
(A) *L. BULGARICUS*

Degree of Sucrose	Fresh Yogurt (cfu/g)	Yogurt Powder (cfu/g)
0	4.3×10^9	1.5×10^7
5%	4.5×10^8	7.0×10^6

10%	8.7×10^8	5.0×10^6
15%	1.0×10^9	1.0×10^6
20%	1.4×10^7	6.9×10^5

(B) <i>S. THERMOPHILUS</i>		
Degree of Sucrose	Fresh Yogurt (cfu/g)	Yogurt Powder (cfu/g)
0	5.3×10^9	1.1×10^7
5%	6.6×10^8	3.6×10^6
10%	1.1×10^9	3.1×10^6
15%	3.7×10^8	1.2×10^6
20%	1.2×10^7	5.1×10^5

1. *L. bulgaricus*



2. *S. thermophilus*

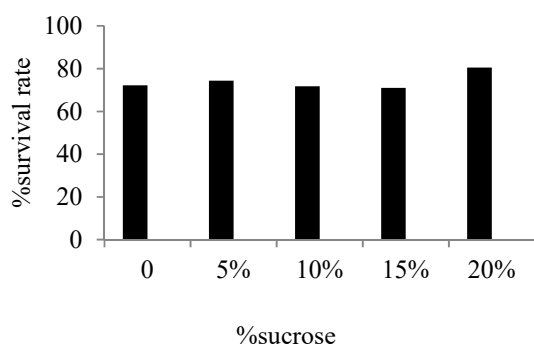


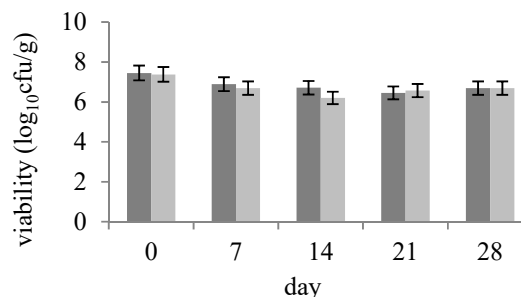
Fig. 2 % survival rate of *L. bulgaricus* and *S. thermophilus* after freeze-dried

C. Effect of Capsule Storage on Viability of *L. bulgaricus* and *S. thermophilus* in Yogurt Powder

Fructo-oligosaccharide (FOS) was added to prebiotic supplement for stimulates growth or activity of bacteria probiotic. FOS may aid survival of *L. bulgaricus* and *S. thermophilus* during freeze-dried and capsule storage. Fig. 3 shows the effect of capsule storage on viability of *L. bulgaricus* and *S. thermophilus* in yogurt powder. The viable cell counts of *L. bulgaricus* and *S. thermophilus*, were about 10^9 cfu/g (before freeze-dried) reduced to 10^7 cfu/g after freeze-died in all yogurt capsule with and without FOS. *L. bulgaricus* and *S. thermophilus* counts in all two yogurt capsules were reduced ($p > 0.05$) almost similar during 28 days of storage. The addition of FOS in yogurt showed no significant effect on the survival of *L. bulgaricus* and *S. thermophilus* compared to their control. This result was similar to other previous studies [4]. The fructo-oligosaccharide may not have been able to protect cell from freeze (-20°C) injury. Oxygen toxicity may be

another factor causing cell death during storage due to oxygen permeation through hard gelatin capsule can be especially problematic for anaerobic bacteria. Moreover, possibilities that lower water in yogurt powder and lower storage temperature may play a more important role in retaining viability of *L. bulgaricus* and *S. thermophilus* in yogurt powder.

1. *L. bulgaricus*



2. *S. thermophilus*

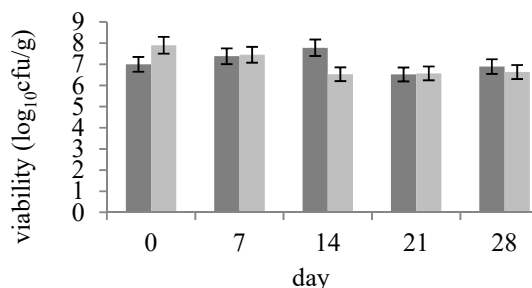


Fig. 3 Change in bacterial counts of *L. bulgaricus* and *S. thermophilus* in capsule during 28 days refrigerated storage (4°C). ■ No probiotic, ■ FOS. T-test showed a significant effect at 5% level during all periods of storage

IV. CONCLUSION

Based on the results of this study, an inoculum size of commercial yogurt starter at 8ml ensured the highest viable cell counts of *L. bulgaricus* and *S. thermophilus* after fermentation of milk. Prebiotic (FOS) had a negative effect on their viability in freeze-dried yogurt (yogurt powder) and during capsule storage for 28 days at 4°C .

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