CHAPTER 5
CONCLUSION AND RECOMMENDATION

This study could be concluded as follow:

1. The physical properties of gelatinized TSB including, structure, pore size, porosity and adsorption property were modified by two freezing methods: quick freezed at -176°C (QF), 5-10 min by liquid nitrogen or slow freezed at -20°C (SF), 24 h in frozen room and followed by drying in freeze-drying system. The SF-FDTB had a puffy surface with large open pore (ca 57.04 μm, diameter) whereas the QF-FDTB had smooth and some part of closed surface area with the smaller pore size (ca 11.18 μm, diameter) for all three commercial brands of Golden Chef®, Special Sacoo® and Thaiworld®. SF-FDTB assisted higher bifidobacterial cells load than QF-FDTB for all tested commercial brands.
2. The suitable immobilization time of *B. longum*, *B. bifidum* and *B. infantis* in QF- and SF-FDTB was 16-18 h at 4-5°C.
3. Special Sacoo®SF-FDTB was suitable for immobilization of *B. longum*, *B. bifidum* and *B. infantis* because of high cell-load and the cheapest price. The maximum immobilization of *B. longum*, *B. bifidum*, and *B. infantis* Special Sacoo®SF-FDTB were 2.6×10^9, 3.9×10^9, and 8.4×10^8 cells per bead, respectively.
4. The procedure for immobilization of *B. longum*, *B. bifidum* and *B. infantis* in Special Sacoo®SF-FDTB are: gelatinizing of tapioca starch beads, freezing the beads at -20°C for 24 h in frozen room,

followed by drying in freeze-drying system and immobilizing with bifidobacterial cell suspension at 4-5°C for 16-18 h, respectively.

5. After immobilization, the beads were then drying in freeze-drying system, the reduction of viable counts after freeze-drying of immobilized *B. longum*, *B. bifidum* and *B. infantis* was ca 1 log-cycle. The viable counts of freeze-dried-immobilized *B. longum*, *B. bifidum* and *B. infantis* Special Sacoo®SF-FDTB were 9.13±0.13, 8.85±0.23, and 8.81±0.12 log CFU/mL, respectively.
6. Immobilization in SF-FDTB with or without the combination of edible bilayer films increase the survival of bifidobacteria in simulated gastrointestinal fluid without enzyme at 37°C for 310 min. The viable counts of free cells of tested bifidobacteria were lower than that of non-coated and coated-immobilized bifidobacteria ca 1-2 log-cycle. The efficiency of immobilization and bilayer coating decreased after storage the samples in pasteurized and sterilized yogurt at 4-5°C for 4 wk.
7. Coating immobilized beads with bilayer films from sodium caseinate and fat (palmitic acid, PANODAN® and beeswax) did not show the significant difference to protect the tested bifidobacteria during storage in pasteurized yogurt at 4-5°C for 4 wk and during incubated in simulated gastrointestinal fluids at 37°C for 310 min when compared to non-coated-immobilized beads.
8. Free cells and immobilized *B. longum*, *B. bifidum* and *B. infantis* could survive in sterilized yogurt during storage time at 4-5°C for 4 wk with the high viable counts of more than 6 log CFU/mL. Whereas the survival of free cells and immobilized-bifidobacteria were 3 and 3-5 log CFU/mL, respectively in simulated gastrointestinal fluids without enzyme at 37°C for 310 min.

Recommendations for future research

1. Coating dried-immobilized beads with edible bilayer films would be recommended to prevent cells from oxygen exposure during storage and use. The open porous structure of SF-FDTB could allow oxygen to enter.
2. Determination the effects of plasticizer on the flexibility and oxygen barrier of sodium caseinate is recommended to eliminate cracking of dried sodium caseinate on the surface of SF-FDTB.
3. Investigation of the other effective coatings would be recommended to improve the survival of bifidobacteria.
4. *In vivo* study the effect of human gastrointestinal tract on the viability of non-coated and bilayer coated-immobilized bifidobacteria in SF-FDTB would be useful to confirm the *in vitro* results from this experiment.
5. Stability of non-coated and coated-dried-immobilized bifidobacteria in SF-FDTB should be evaluated to determine the appropriated storage condition and shelf life.
6. Immobilization of bifidobacteria in cereal grains should be studied to improve the survival rate of bifidobacteria.