

**Title :** Development of Synbiotic Formulation Method via Freeze Drying Technique: Part 2

**Author(s) :** Apitchaya Saejang **Student ID :** 640510193

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**Advisor(s) :** Associate Professor Dr. Apinun Kanpiengjai

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## ABSTRACT

Synbiotics are a combination of prebiotics and probiotics, which are beneficial microorganisms for health. Neokestose is one of the smallest fructooligosaccharide (FOS)-type prebiotics, but it has distinctive structure from other prebiotics in the same group, such as 1-kestose and 6-kestose. Several studies revealed that neokestose is more stable and more resistant to enzymatic digestion in the human gastrointestinal tract, as well as capable of stimulating the growth of bifidobacteria and lactobacilli. Using neokestose as the sole prebiotic may not be sufficient to stimulate human gut microbiota. Therefore, utilizing neokestose in the form of a synbiotic may enhance the prebiotic properties of neokestose. This study aimed to develop synbiotic powder of neokestose and four strains of probiotic lactic acid bacteria including *Limosilactobacillus (Lm.) fermentum* FS2.2, *Lm. fermentum* FS48.1, *Lactobacillus argenteratensis* FS41.1, and *Enterococcus faecium* CG403. The basic formulation consisted of three components including whey protein isolate (WP), which is served as a drying aid, neokestose, and probiotic lactic acid bacteria ( $10^8$  cells/mL). Different formulae were made by varying concentrations of whey protein isolate at 10 and 20 (%w/v) and neokestose at 0, 5, 10, 15, and 20 (%w/w), resulting in 10 formulae including WP10N0, WP10N5, WP10N10, WP10N15, WP10N20, WP20N0, WP20N5, WP20N10, WP20N15, and WP20N20. All formulae were freeze-dried prior to determining survival of all probiotics using drop plate method. The Principal Component Analysis indicated that the formulae WP10N0, WP20N0, and WP20N5 exhibited similar patterns in %survival to each other, yet they were categorized in the same group. On the other hand, other formulae demonstrated variability in %survival depending on the probiotic strains. The results obtained from analysis of variance and multiple comparison of the mean values of %survival showed that the formulation WP10N20 significantly provided the highest %survival for all probiotic strains ( $p < 0.05$ ). This formulation was therefore considered the most suitable for developing synbiotic products. Additionally, the findings indicated that neokestose may possess cryoprotective properties.

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