CHAPTER 3

MATERIALS AND METHODS

3.1 Microorganisms

Lactobacillus fermentum strains 2311M was isolated from Thai traditional fermented foods (miang), which was obtained from Faculty of Science, Maejo University, Thailand (Srikanjana et al., 2008). *Lactobacillus casei* sub.sp. *paracasei* F-19 purchase from Medipharm and *Lactobacillus plantarum* V299 purchase from Wiesby. There strain were stored at -80°C in MRS medium containing glycerol stocks before used in any experiments.

One loop of each probiotic microorganism from glycerol stocked at -80 °C was inoculated in 5 ml. of MRS broth and incubated at 37°C for 24 hours. 0.5 ml culture transfer to 5 ml MRS broth and incubated at 37°C for 24 hours and use as starter culture.

Growth curve of probiotic microoraganisms was study using five milliliters of starter cultured were transferred to 95 ml. of MRS broth. Three milliliters were collected every hour until reached 12 hours for *L. fermentum* 2311M, 29 hours for *L. casei* sub.sp. *paracasei* F-19 and 18 hours for *L. plantarum* V299 to measure for an optical density at 600 nm by using spectrophotometer. The data were plotted as a graph and showed in appendix D.

3.2 Preparation of probiotic inoculum

Transferred 5 ml of starter culture into 95 ml MRS broth and incubated at 37°C until harvesting time. The fresh cell of each probiotic microorganism was obtained from log phase, at 9 hours for *L. fermentum* 2311M, 18 hours for *L. casei* sub.sp. *paracasei* F-19 and 12 hours for *L. plantarum* V-299, by centrifugation at 6,000 rpm,4°C for 15 minutes. The collected cell was resuspended in 1 ml. of 0.85% NaCl solution.

3.3 Evaluation of viable cell

3.3.1 Evaluation viable cell number before drying

The fresh cell of probiotic were inoculated in 500 ml of skim milk solution. The sample was collected 5 ml. to analyze for the viability of probiotic bacteria before drying. Then, 0.1 ml of the diluted sample was spreaded on GYP with bromocresol purple and NaN_3 by using a spreader. The agar was incubated at 37°C for 3 days. The probiotic bacterial count was reported in cfu/g.

3.3.2 Evaluation viable cell number after drying

The number of viable cells was counted on GYP agar plate. Each sample of dried probiotic 1 g was rehydrated with 9 ml of 0.85% NaCl solution by vigorous shaking for 2 min. The rehydrated sample was serially diluted in 0.85% NaCl solution and then, 0.1 ml of the diluted sample was spreaded on GYP with bromocresol purple and NaN_3 by using a spreader. The agar was incubated at 37°C for 3 days. The probiotic bacterial count was reported in cfu/g.

3.3.3 Calculaton of percentage of cells survival

The percentage of cells survival at each of the air outlet temperatures tested was calculated as follow: % survival = $(N/N_0) \times 100$, where N_0 is the number of bacteria per gram of dry matter before drying and N is the number of bacteria per gram of dry matter in the powder (Gardiner et al, 2000).

3.3.4 Analysis moisture content of product

Moisture content of dried powders was determined in by Infrared Moisture Determination Balance FD-620 (KETT ELECTRIC LABORATORY, Japan)

3.4 Study on survival rate and moisture content of probiotic bacteria in skim milk solution for spray drying.

3.4.1 Selection air outlet temperatures

This study focus on condition in spray drying process especially, the

air outlet temperature is effect to quality of product. So, investigate the effect of difference air outlet temperature at 65°C, 75°C and 85°C and used constant of atomizing air pressure at 15 kg/cm^2 and feed rate at 25 ml/min.

Table 3.1 Condition of spray drying.

Air outlet temperature (°C)	Air inlet temperature (°C)	Atomizing air pressure (kg/cm2)	Feed rate (ml/min)
65	100-120	15	25
75	130-140	15	25
85	150-160	15	25

3.4.2 Preparation of skim milk solution with probiotic bacteria for spray drying

1. Five grams of probiotic cell were inoculated in 500 ml skim milk solution after autoclave at 110°C, 10 min for twice. The sample was collected 5 ml. to analyze for the viability of probiotic bacteria before drying (follow at 3.3.1).

2. The spray drying cultures were directly spray dried with a spray Dryer. Which condition showed in table 3.1 Sample of dried probiotic was analyzed for viable cell number after drying (followed at 3.3.2).

3. Sample of dried probiotic was analyzed for cell survival and moisture content of product (followed at 3.3.3 and 3.3.4).

3.4.3 Variation of starter probiotic cells in skim milk solution for spray drying.

1. Increase the starter probiotic cells from 5 g to 10 g and 15 g were inoculated in 500 ml skim milk solution. The samples were collected 5 ml. to analyze for the viability of probiotic bacteria before drying (follow at 3.3.1).

2. The spray drying cultures were directly spray dried with a spray dryer. Which condition is 75°C of air outlet temperature, 130-140°C of air inlet temperature, 15 kg/cm^3 of atomization air pressure and feed rate 25 ml/min.

3. Sample of dried probiotic was analyzed for viable cell number after drying (followed at 3.3.2).

4. Analyzed for survival rate and moisture content after drying (followed at 3.3.3 and 3.3.4).

5. The dried samples were kept in different packaging (plastic Zip bags and aluminum bags) and stored at 4°C in refrigerator and room temperature for 30 days. The samples were collected every 15 days to analyze for probiotic bacterial count and moisture content.

3.5 Study on survival rate and moisture content of probiotic bacteria in whipping cream solution for spray drying.

1. 75 g of whipping cream powder was mixed in 425 ml of cold water after that, 5 g of probiotic cell was added.

2. The sample was collected 5 milliliters to analyze for the viability of probiotic bacteria before drying (follow at 3.3.1).

3. The spray drying cultures were directly spray dried with a spray dryer. Which condition is 75°C of air outlet temperature, 130-140°C of air inlet temperature, 15 kg/cm^3 of atomization air pressure and feed rate 25 ml/min.

4. Sample of dried probiotic was analyzed for viable cell number after drying (followed at 3.3.2).

5. Analyzed for survival rate and moisture content after drying (followed at 3.3.3 and 3.3.4).

6. The dried samples were kept in different packaging (plastic zip bags and aluminum bags) and stored at 4°C in refrigerator and room temperature for 30 days. The samples were collected every 15 days to analyze for probiotic bacterial count and moisture content.

3.6 To monitor packaging material to keep probiotic added skim milk and whipping cream powder during storage at different storage temperatures.

Dried samples of skim milk and whipping cream probiotic were also kept in plastic zip bags or aluminum bags and stored at 4°C in refrigerator and room temperature for 30 days to investigate their stability viable cell and moisture content (followed at 3.1.6). The sample was collected every 15 days to analyze for probiotic bacterial count. One gram of the dried sample probiotic was diluted in 0.85% NaCl solution, 9 ml. Mixed by the vortex. Then, 0.1 ml of the diluted sample was spreaded on GYP with bromocresol purple and NaN_3 by using a spreader. The agar was incubated at 37°C for 3 days. The probiotic bacteria was enumerated in cfu/g and calculate the percentage of cell survival.