



**APPENDICES**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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**APPENDIX A**

**Reagents and Media**

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**APPENDIX A****Reagents and Media****Lactic acid bacteria (LAB) media recipes.**

## 1. MRS medium

1. Dissolve the following in 850 ml of distilled water.
  - 10 g Gelatin
  - 8 g Beef extract
  - 4 g Yeast extract
  - 18.5 g Glucose
  - 1 g Tween-80
  - 2 g  $K_2HPO_4$
  - 3 g Na-acetate
  - 2 g  $(NH_4)_2$  citrate
  - 0.2 g  $MgSO_4 \cdot 7H_2O$
  - 0.05 g  $MnSO_4 \cdot H_2O$
  - 0.04 g Bromocresol purple
2. Adjust pH to between 6.2 and 6.5.
3. Bring to 1000 ml with distilled water.
4. Autoclave at 121°C for 15 min or filter sterilize.

## 2. GYP agar plate

- 10 g Glucose
- 10 g Yeast extract
- 10 g Peptone
- 5 ml Salt solution (see below)
- 0.04 g Bromocresol purple
- 0.2 g  $\text{NaN}_3$
- 1000 ml Distilled water

### Stock salt solution :

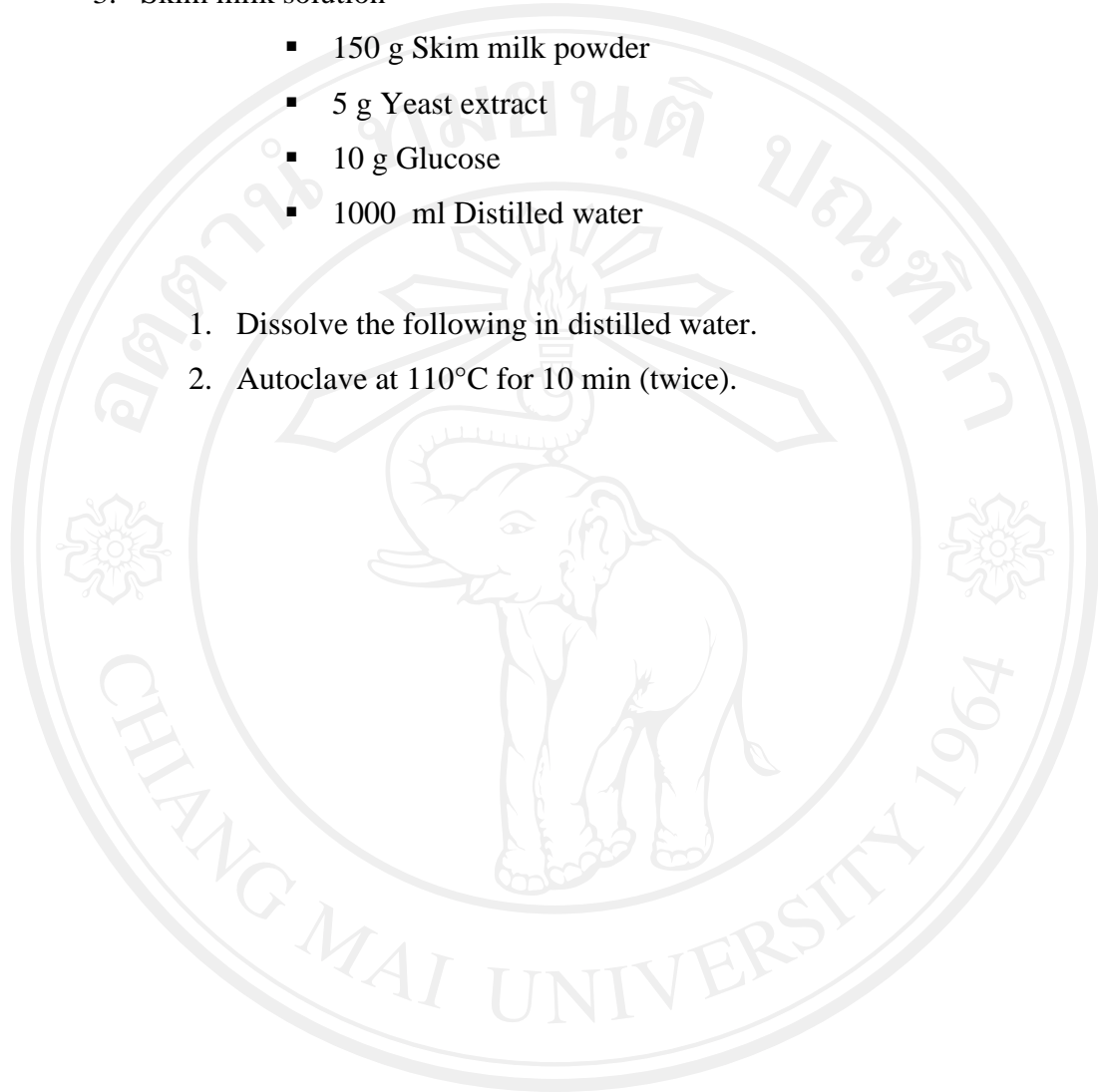
- 40 g  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$
- 2 g  $\text{MnSO}_4 \cdot 4 \text{H}_2\text{O}$
- 2 g  $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$
- 1000 ml Distilled water

1. Dissolve the following in distilled water.
2. Add 15 g/l agar before autoclaving.
3. Autoclave at  $121^\circ\text{C}$  for 20 min.
4. Let cool to  $\sim 55^\circ\text{C}$  and add desired antibiotics at this point. Pour into 10 cm Petri plates. Let the plates harden, then invert, and store at  $+4^\circ\text{C}$ .

### 3. Skim milk solution

- 150 g Skim milk powder
- 5 g Yeast extract
- 10 g Glucose
- 1000 ml Distilled water

1. Dissolve the following in distilled water.
2. Autoclave at 110°C for 10 min (twice).



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### Reagents recipes

#### 1. Stock Sodium Chloride (NaCl) 0.85%

- 0.85 g NaCl
- 100 ml Distilled water

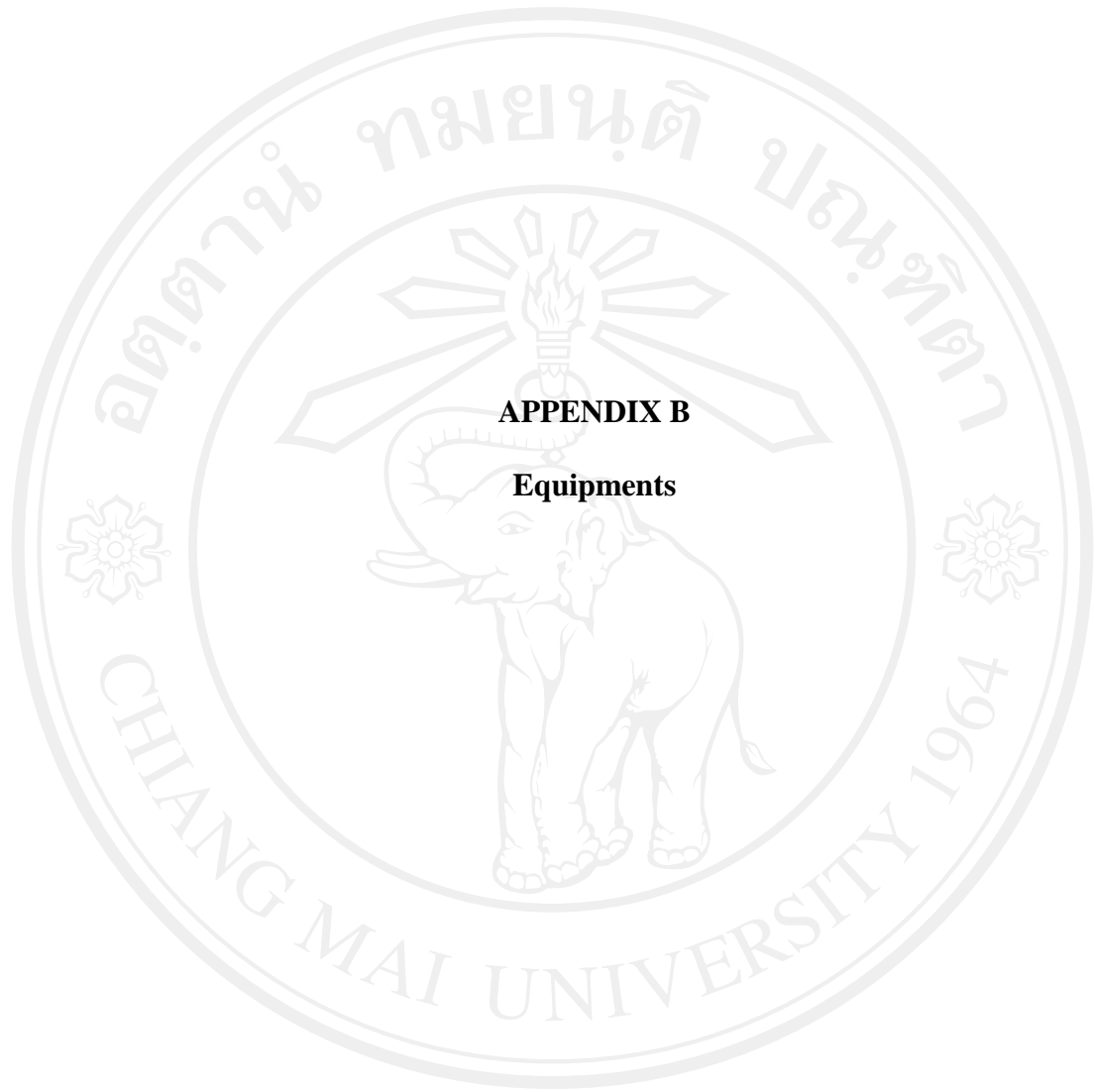
1. Dissolve the following in distilled water.
2. Autoclave at 121°C for 15 min.

#### 2. Glycerol stock solution

- 650 ml Glycerol
- 12.048 g MgSO<sub>4</sub>·7H<sub>2</sub>O
- 3.0285 g Tris-HCl
- 1000 ml Distilled water

Tris-HCl :

1. To make 1 mol/L Tris-HCl dissolve 121.1 g of tris base in 700 ml of double distilled water.
2. Bring to desired pH with concentrated HCl<sub>aq</sub> (usually 7.5 or 8.0).
3. Add double distilled water to 1 L.
4. Filter with 0.5 µm filter.
5. Autoclave.
6. Store at room temperature.



**APPENDIX B**

**Equipments**

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**APPENDIX B****Spray dryer****Specification**

- Spray dryer manufactured by J.C. Machinery Engineering, Thailand.
- A nozzle atomizer with a length of 44 cm.
- A co-current air flow.
- A blower speed at 38 Hz.

### Infrared Moisture Determination Balance FD-620



#### Specification

- Kett Electric Laboratory, Japan.

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**Aluminum bag for storage.**



**Plastic zip bag for storage.**





**APPENDIX D**

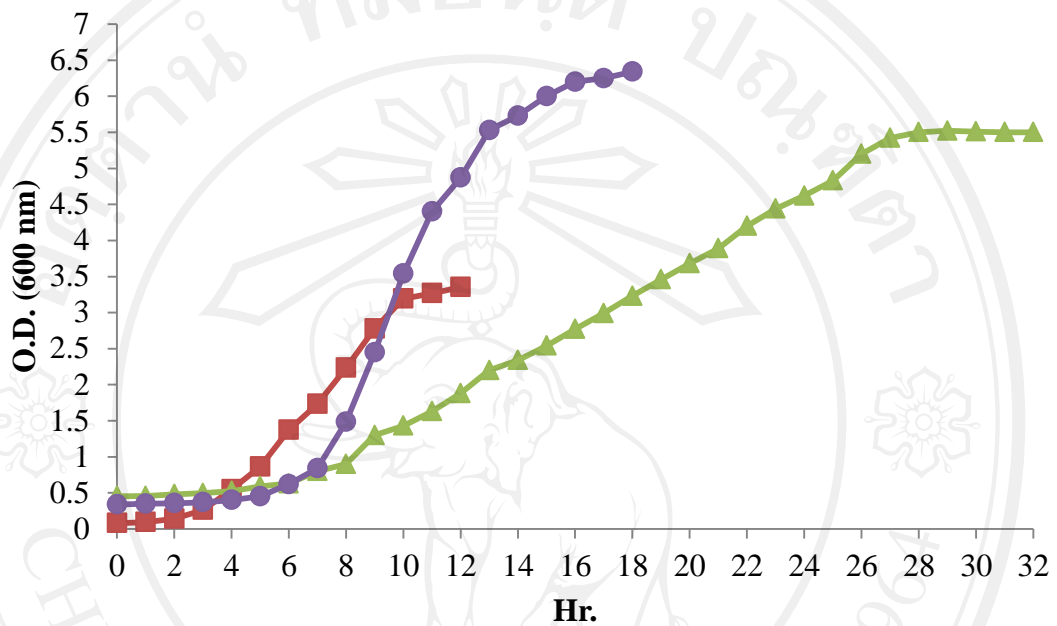
**Growth curve of probiotic microorganisms**

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### Growth curve of probiotic microorganisms



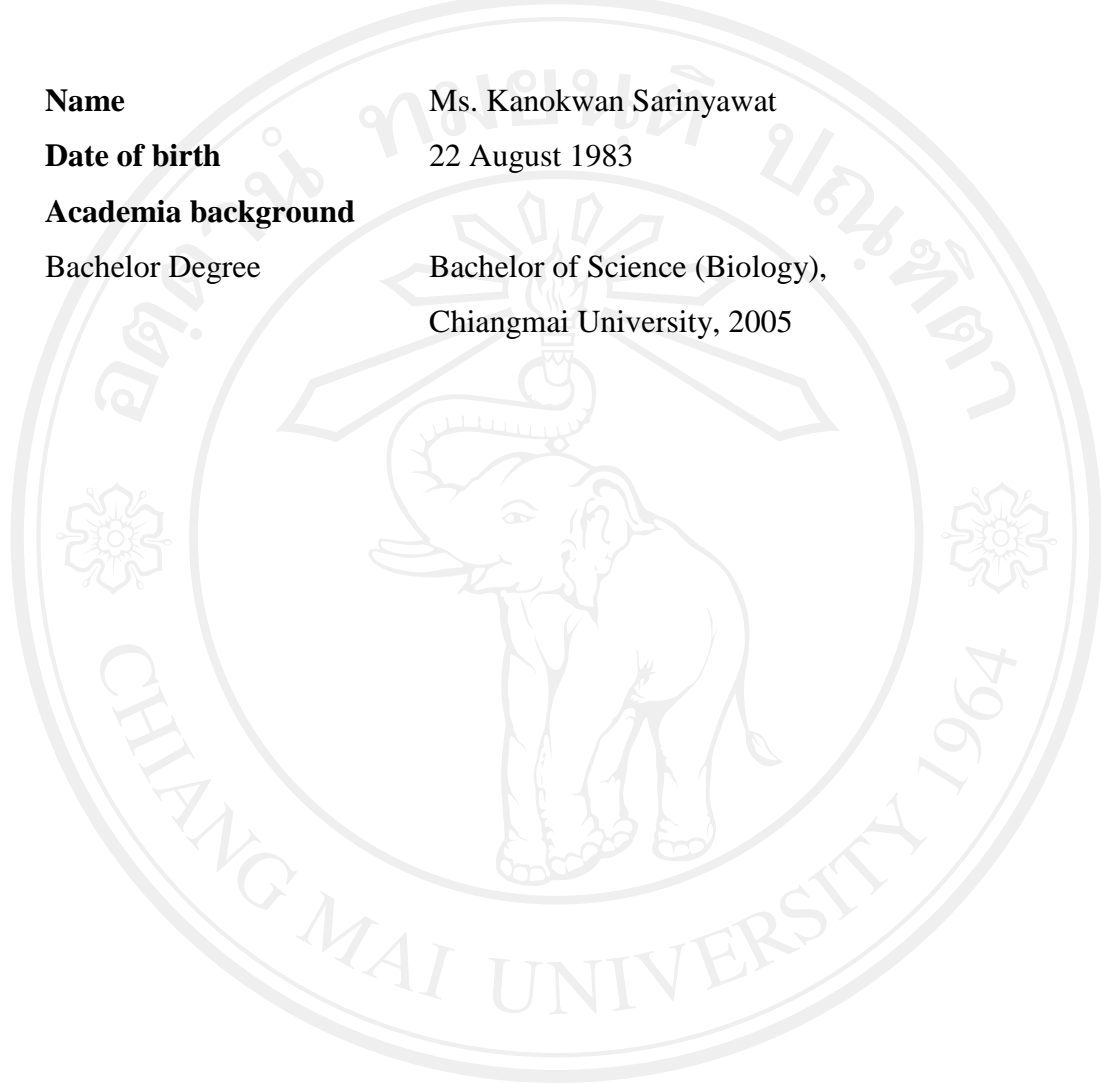
Growth curve of *L. casei* subsp. *paracasei* F-19 (▲)

*L. fermentum* 2311M (■)

*L. plantarum* V-299 (●)

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