

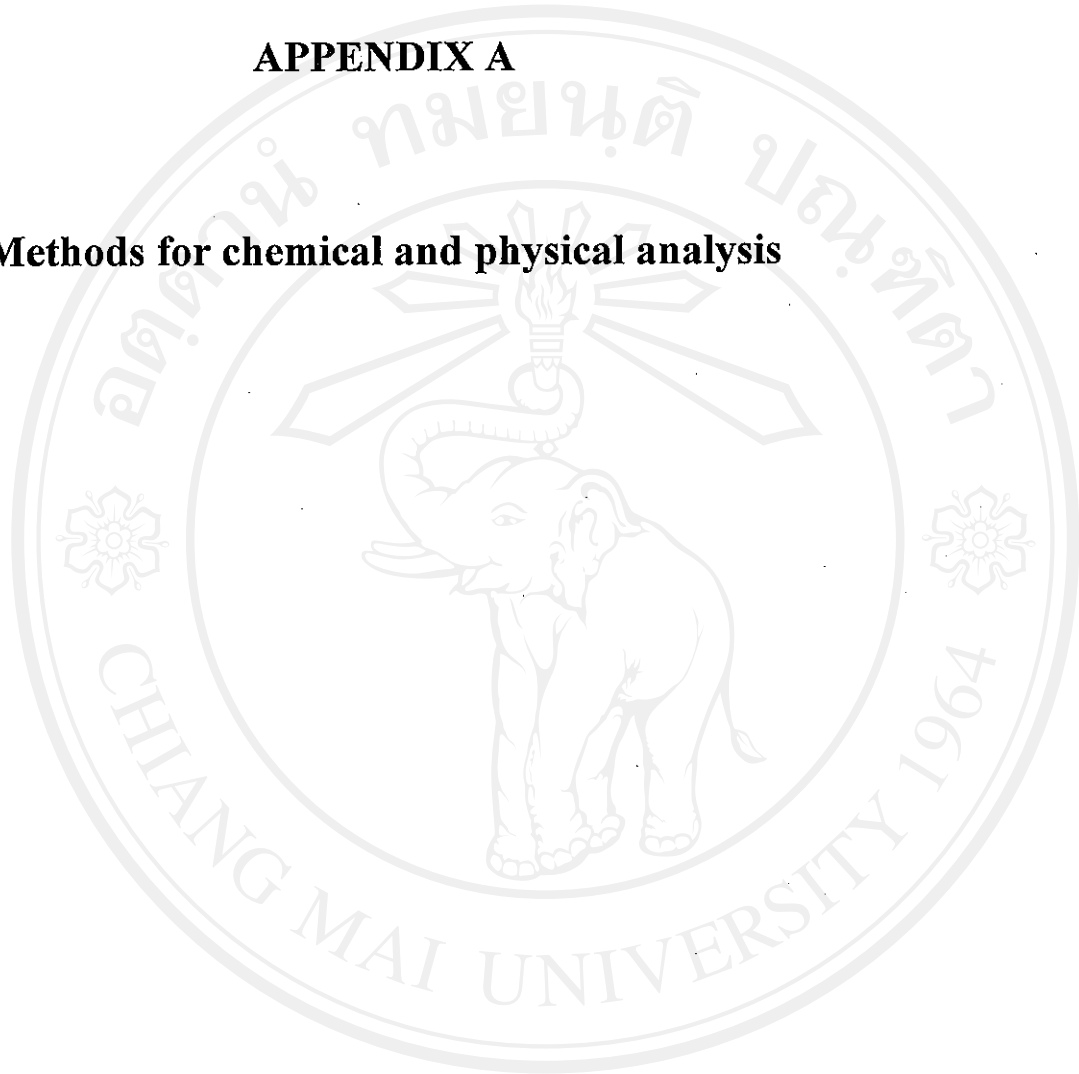
APPENDICES

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University
All rights reserved

APPENDIX A

Methods for chemical and physical analysis



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved

1. Total titratable acidity analysis (AOAC, 2000)

Pipette 10 ml of sample into a 125 ml flask. Drop phenolphthalein 2-3 drops and titrate the sample with 0.1 M NaOH until sample reach the end point (sample solution became pink that was persisted for 30 seconds).

$$\% \text{ lactic acid} = \frac{\text{ml of 0.1 M NaOH} \times 0.1 \times 0.009 \times 100}{\text{Volume of sample}}$$

2. Moisture and Total solid analyses (AOAC, 2000)

Heated an empty moisture dish in a hot air oven about 20-30 minutes. Cool in a desiccator and weigh the dish. Into the cooled and weighed dish (provided with a cover), previously heated to $100 \pm 3^{\circ}\text{C}$, accurately weigh 15-20 g of sample. Uncover the dish and dry the dish with its cover and contents for 3 hours in an oven provided with opening for ventilation and maintained at $100 \pm 3^{\circ}\text{C}$. Cover the dish while it is still in the oven, transfer to a desiccator and weigh the dish soon after it reached a room temperature. Dry the sample again for several times until the sample has a constant weight.

$$\% \text{ moisture content} = \frac{\text{Loss in the sample weight during drying} \times 100}{\text{Initial weight of the sample}}$$

$$\% \text{ total solid} = 100 - \% \text{ moisture content}$$

3. Fat analysis (AOAC, 2000)

Weight sample (0.5 – 1.0 g) and place into a separated funnel. Add 10 ml water and shake. Add 1.25 ml ammonia solution, 10 ml ethyl alcohol and 25 ml diethyl ether, close with a stopper and shake vigorously for 1 minute. Carefully release the pressure of the funnel. Add 25 ml petroleum ether, close the stopper and shake vigorously for 1 minute. Carefully release the pressure. Let stand until an upper liquid is practically clear (~30 minutes). Pour the upper clear solution into a previously weighed beaker. Take the beaker to stand in a hood until diethyl ether and petroleum are evaporated and place the beaker in a hot air oven ($T = 102 \pm 2^\circ\text{C}$) for 2 hours. Cool in a desiccator and weigh the sample.

$$\% \text{ fat content} = \frac{(W_2 - W_1) \times 100}{W_1}$$

W_1 = Weight of sample

W_2 = Weight of beaker and fat

W_3 = Weight of beaker

4. Ash analysis (AOAC, 2000)

Weigh 15-20 g sample into an ashing dish that has been heated, cooled in a desiccator and weighed soon after reaching room temperature. Before ashing the sample, heat the sample on a bunched lamp until no more black smoke appeared. Then ash the sample in a muffle furnace at 550°C until light gray ash results or until it

reaches a constant weight. Cool in a desiccator and weigh soon after reaching room temperature.

$$\% \text{ Ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

5. Fiber analysis (AOAC, 2000)

Weigh 10-20 g sample into a 500 ml beaker. Transfer 1.25 M sulfuric acid (200 ml) into the beaker. Boil the sample solution on a hot plate for 30 minutes. Filter the sample solution using a Whatman filter paper no. 4 until it dries by applying a vacuum pump and wash the residue with boiling water until the sample does not have acid (do a test using a litmus paper). Place 200 ml of 1.25% NaOH into a beaker and boil the beaker on a hot plate. Wash the residue on the filter paper with distilled water. Boil the sample again on the hot plate for 30 minutes. Filter the sample using a Whatman filter paper no. 4 and wash the residue with boiling water. Transfer the filter paper with the sample residue into a crucible and dry at $102 \pm 2^\circ\text{C}$ for 3 hours. Cool in a desiccator and weigh. Then ash the residue for 2 hours at $550 \pm 10^\circ\text{C}$, cool in the desiccator and weigh.

$$\% \text{ Crude fiber} = \frac{(W_4 - W_3 - W_2) + (W_5 - W_3)(100 - \% \text{H}_2\text{O} - \% \text{fat})}{W_1}$$

W_1 = Weight of sample

W_2 = Weight of filter paper

W_3	=	Weight of crucible
W_4	=	Weight of crucible + filter paper + sample after drying
W_5	=	Weight of crucible + ash
%H ₂ O	=	Moisture content of sample
%fat	=	Fat of sample

6. Carbohydrate content (AOAC, 2000)

Carbohydrate content was determined by measuring the difference of the original sample minus the moisture, protein, crude fat and mineral contents calculated at the same moisture level.

7. Protein analysis (AOAC, 2000)

Place weighed sample (5-10 g) in a digestion flask. Add 8 g catalyst mixture and 20 ml H₂SO₄. Place the flask in an inclined position in a digestion machine and heat the machine gently until frothing ceases. Continue boil briskly until the solution clears (~2 hours).

Cool, add distilled water to dilute the mixture solution and pour into a distilling flask. Add 400 ml H₂O (ammonia-free water) and a few Zn granules to prevent bumping. Immediately immerse a condenser tip into a receiver that contains 50 ml of 2% boric acid solution in a 500 ml flask and 5-7 drops indicator. Add 75 ml of 50% sodium hydroxide using a funnel into the distilling equipment. Rotate the distilling flask to mix the contents thoroughly; then heat until all NH₃ has been

distilled (≥ 150 ml distillate). Remove the receiver, wash the tip of the condenser and titrate excess standard acid in distillate with 0.1 M H_2SO_4 . Do blank determination to correct any nitrogen content in reagents.

$$\% \text{N} = \frac{(V_a - V_b) \times N_{\text{H}_2\text{SO}_4} \times 1.4007}{W}$$

V_a = ml of standard acid for sample titration

V_b = ml of standard acid for blank titration

$N_{\text{H}_2\text{SO}_4}$ = normality acid

W = weight of sample (g)

$$\% \text{ Protein} = \% \text{ N} \times \text{factor} \quad (\text{a factor value} = 6.25 \text{ for mung bean})$$

8. Viscosity by a Brookfield viscometer (Brookfield, England)

Viscosity of bean milk was measured using a Brookfield viscometer. The needle S18 was used in this measurement. An amount of 8 ml bean milk was poured into a special container and placed in the correct position under the viscometer. The viscosity value was recorded 30 seconds after inserting the viscometer needle.

Measurements of bean milk samples were conducted every 7 days storage at $25 \pm 2^\circ\text{C}$.

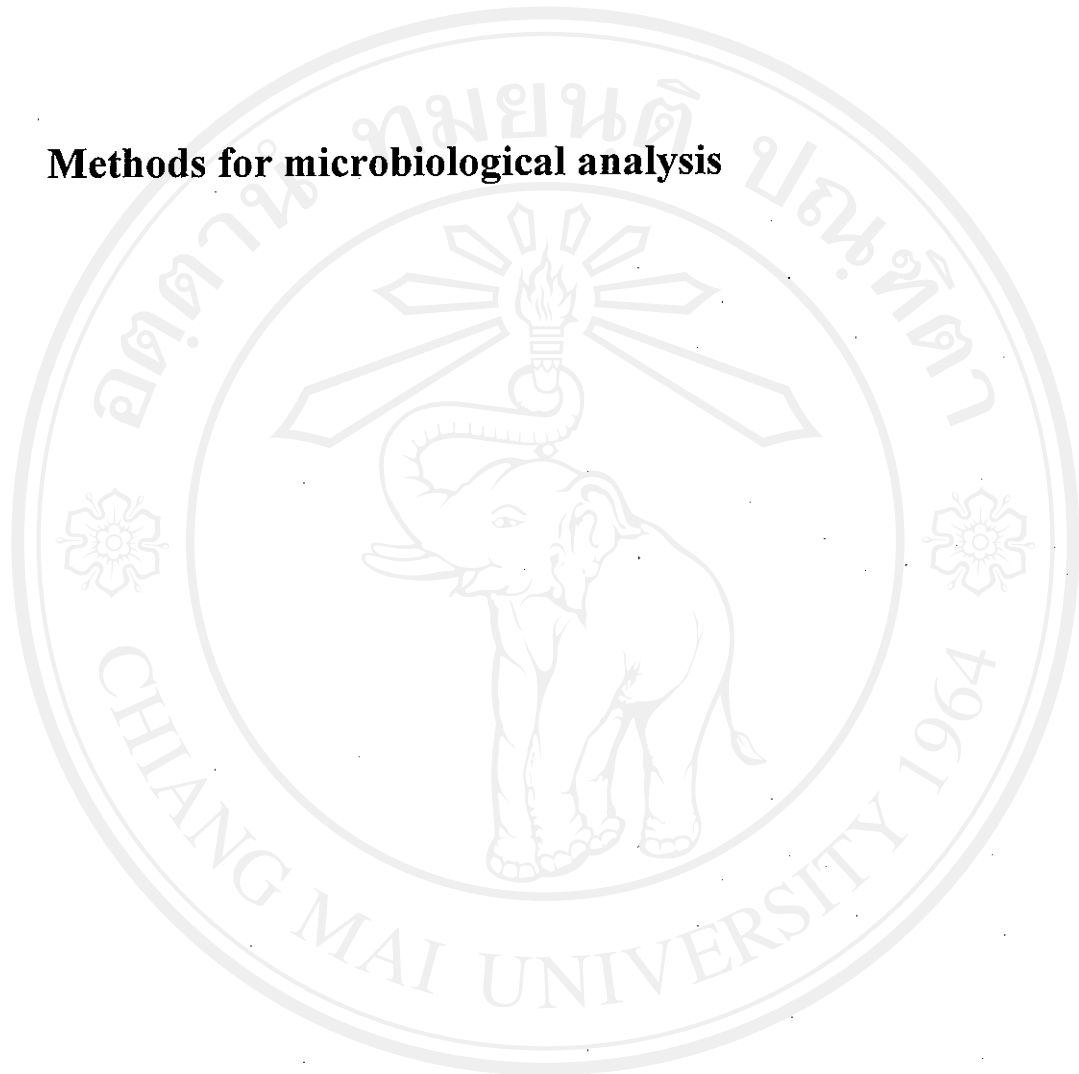
All rights reserved

9. Color analysis by a colorimeter (Minolta, Japan)

CIE L*, a* and b* values of bean milk samples were measured by a colorimeter (Minolta CR-300, Japan). Samples of bean milk were prepared by pouring 30 ml of bean milk into a white plastic cup. The colorimeter probe was then dipped into the bean milk samples and the L*, a* and b* values that were shown by the colorimeter were recorded. The colorimeter was calibrated against a standard white tile prior to the bean milk measurement and measurements were conducted every 7 days storage at $25 \pm 2^{\circ}\text{C}$ (Kritsawan, 2006).

APPENDIX B

Methods for microbiological analysis



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University
All rights reserved

1. Gram strain (คณาจารย์จุลชีววิทยา, 2544)

1.1 Crystal violet stain

Crystal violet (Gentian violet)	0.5 g
Distilled water	100 ml

1.2 Gram iodine solution

Iodine	1.0 g
Potassium iodine	2.0 g
Distilled water	300 ml

1.3 Decolourizer

95% Alcohols	250 ml
Acetone	250 ml

1.4 Safranin O stain

Safranin O	2.5 g
95 % Alcohols	100 ml

Dilute with distilled water to 5-10 fold before used.

Gram stain procedure (Barnett *et al.*, 1997)

1) Use only thoroughly dried and heat-fixed smears for the Gram stain.

Cover a cooled slide with crystal violet and stain for 1 minute.

2) Quickly and gently rinse off the dye with water.

3) Apply Gram iodine. Leave it on the smear for 1 minute.

4) Quickly and gently rinse off the mordant with water.

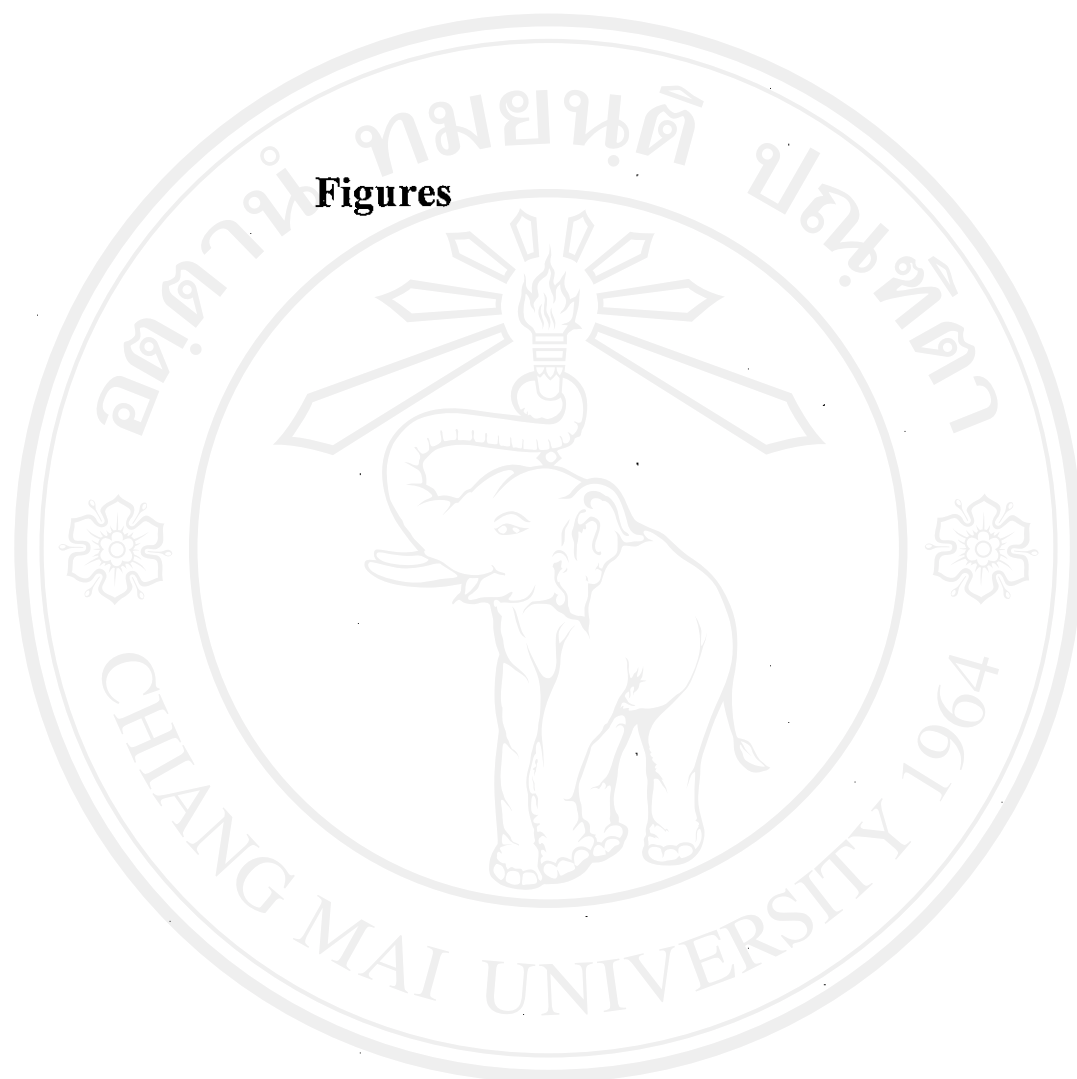
5) Decolorize the smear for less 1 second.

- 6) Counterstain with safranin O for 1 minute. Rinse the slide gently and briefly with water. Gently blot the slide dry or allow it to air dry.
- 7) Examine slide with a light microscope.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

APPENDIX C

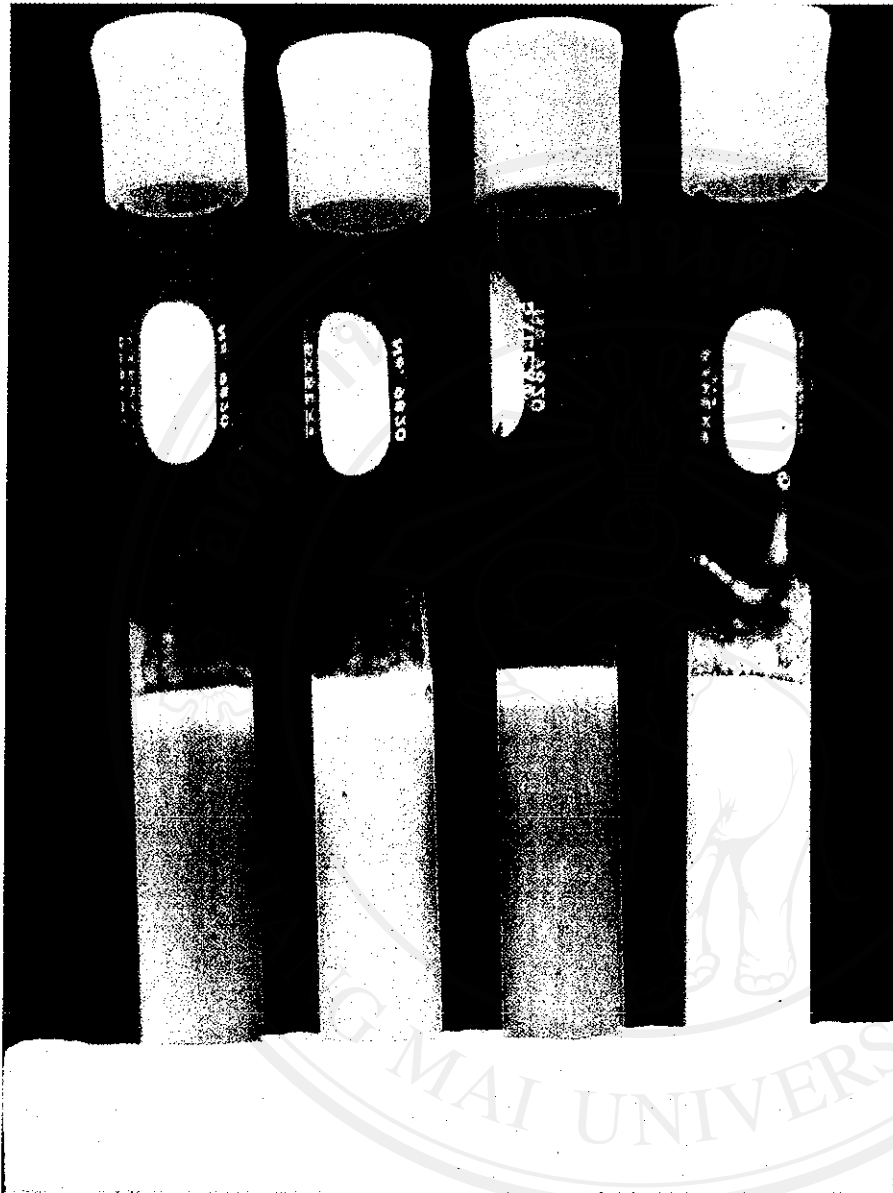


Figures

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved



(a)

(b)

(c)

(d)

Figure C1 Different types of bean milk, including a) black bean milk, b) mung bean

milk, c) red bean milk and d) soy bean milk

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

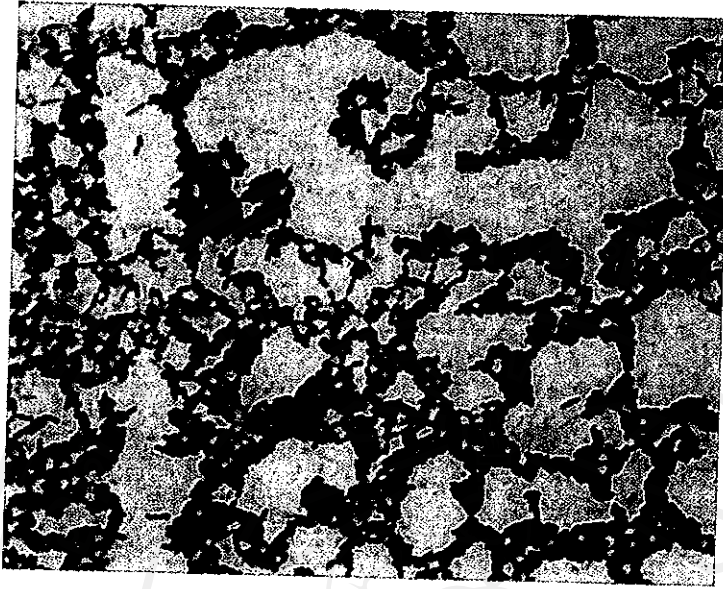


Figure C2 Gram staining of *Lactobacillus acidophilus* TISTR 450 (1,000x magnification under a light microscope)



Figure C3 A calcium-alginate bead contained *Lactobacillus acidophilus* TISTR 450 cells

Curriculum Vitae

- Name:** Miss Chiraphorn Sankonkit
- Date of birth:** 23/04/1979
- Academic background:**
- Dara Academy School Chiang Mai, 1985-1997
 - Faculty of Associated Medical Sciences, Major of Medical Technology, Chiang Mai University, Chiang Mai , B.S. (Medical Technology) 1997-2001
- Work Experiences**
- Medical Technologist at Division of Pulmonary , Critical Care and Allergy, Department of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand (2001- 2004)
 - Medical Technologist at Research Institute for Health Sciences, Chiang Mai University, Chiang Mai 50200, Thailand (2001- at present).