

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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Appendix A-1: ABT-5 (Probiotic Cultures)

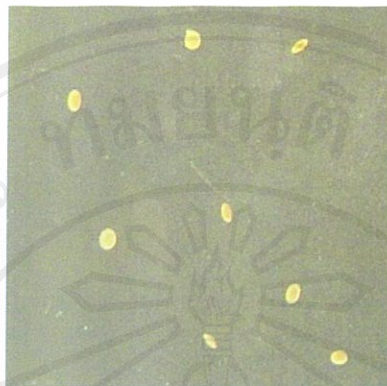


Figure A-1 Colonies of *Streptococcus thermophilus* on M17 Agar.



Figure A-2 Colonies of *Lactobacillus acidophilus* on HHD Agar.



Figure A-3 Colonies of *Bifidobacterium bifidum* on HHD Agar.

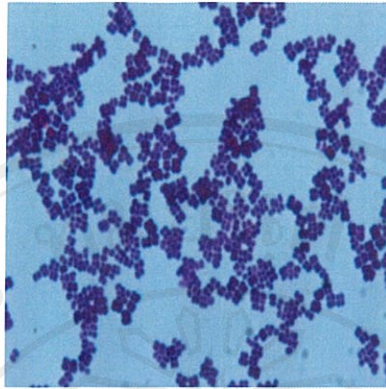


Figure A-4 Gram staining of *S. thermophilus* using a magnification of 1,000.

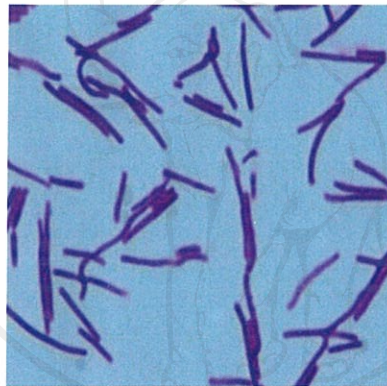


Figure A-5 Gram staining of *L. acidophilus* using a magnification of 1,000.

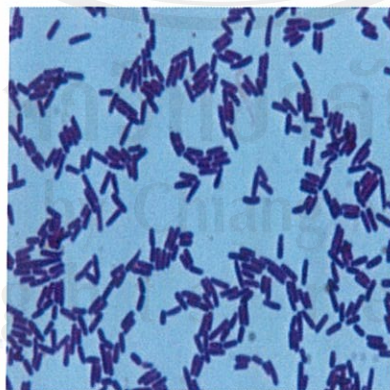


Figure A-6 Gram staining of *B. bifidum* using a magnification of 1,000.

Appendix A-2: Green Soya Bean Yoghurt Ice Cream



Figure A-7 Frozen green soya bean.

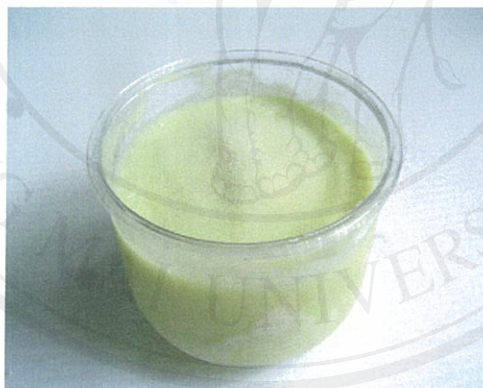


Figure A-8 Green soya bean yoghurt ice cream.

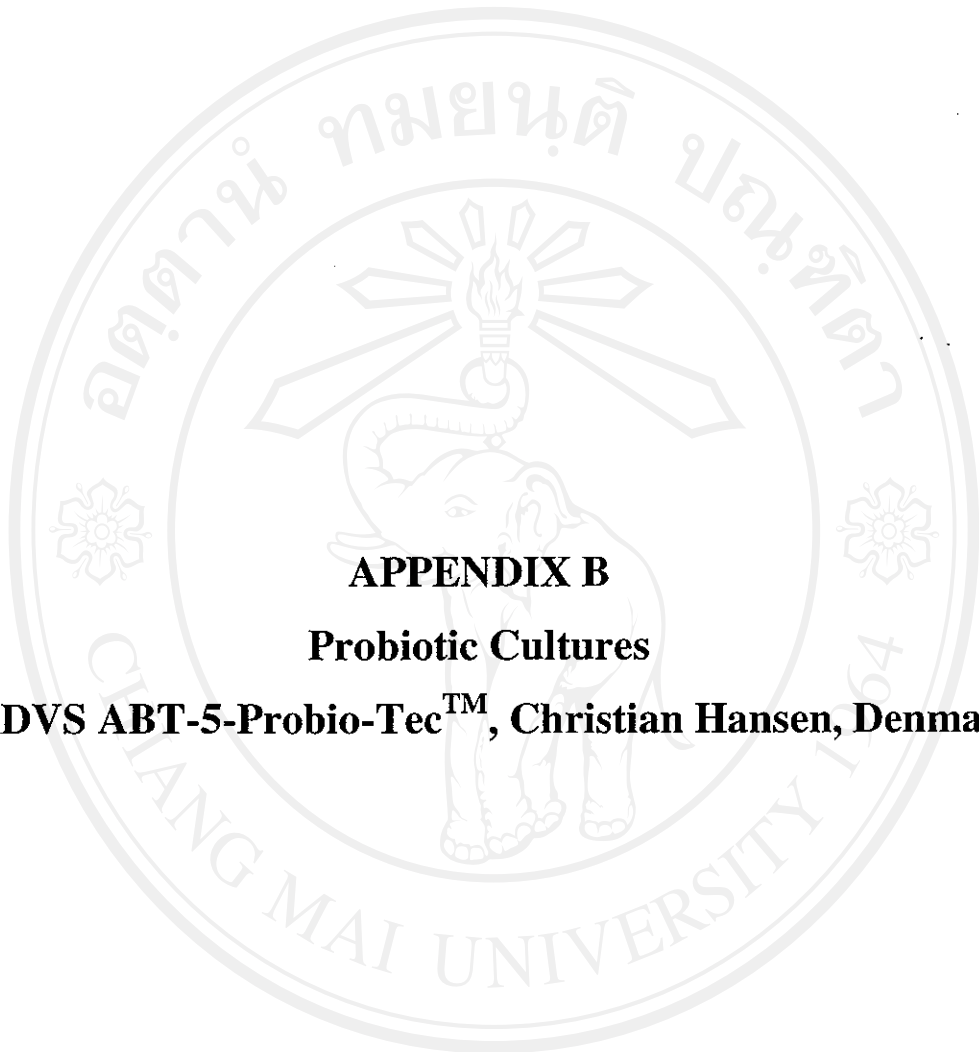
Appendix A-3: Equipment



Figure A-9 An electric juice blender.



Figure A-10 An ice cream machine.



APPENDIX B

Probiotic Cultures

(FD-DVS ABT-5-Probio-Tec™, Christian Hansen, Denmark)

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Appendix B-1: Product Information

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Description	Thermophilic Lactic Culture. Defined mixed strain culture containing <i>Lactobacillus acidophilus</i> LA-5, <i>Bifidobacterium bifidum</i> BB-12 and <i>Streptococcus thermophilus</i> . The probiotic strains in this culture have a long history of safe use. ABT-5 is supplied in a convenient freeze-dried form.
Application	The culture will produce a fermented milk with medium body and mild flavor and a minimal post-acidification. ABT-5 is ideal for the manufacturing of the following types of fermented milk products: <ul style="list-style-type: none"> • Cup set • Stirred • Drinking
Storage and shelf life	Freeze-dried cultures should be stored at -18°C (0°F) or below. If the cultures are stored at -18°C (0°F) or below, the shelf life is at least 24 months. At +5°C (41°F) the shelf life is at least 6 weeks.
Instructions for use	Remove the cultures from the freezer just prior to use. DO NOT THAW THESE CULTURES. Sanitize the top of the pouch with chlorine. Open the pouch and pour the freeze-dried granules directly into the pasteurized product using slow agitation. Agitate the mixture for 10-15 minutes to distribute the culture evenly.
Dosage	Recommended dosage of FD-DVS ABT-5:

DVS inoculation percentage	Amount of milk to be inoculated			
	1,000 L	5,000 L	500 gallon	1,000 gallon
500U / 2500L** 500U / 660 gallon	200U	1,000U	~400U	~750U

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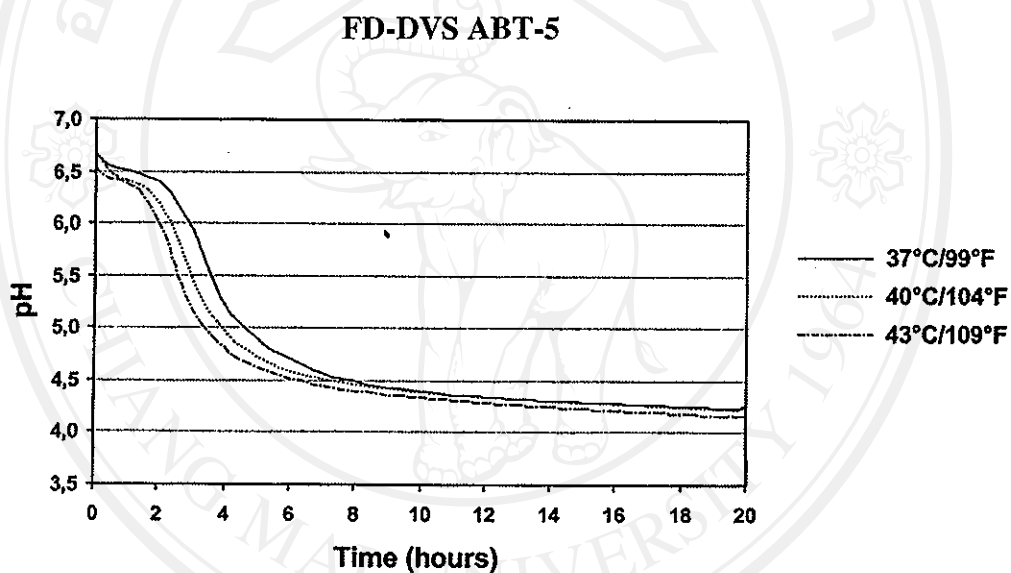
Appendix B-2: Technical Information

CHR HANSEN

Kosher status ABT-5 is Kosher approved (Circle K D) year-round use, excluding Passover.

Technical Information

Figure B-1 The effect of temperature on acidification.



Fermentation conditions:

Lab milk 9.5% T.S.: 140°C/8 sec.-100°C/30 min
500U/2500 L Inoculation

NB: Note that the accuracy of these curves is relative and subject to experimental error.

Technical service Chr. Hansen's worldwide facilities and the personnel of our application and technology center are at your disposal with assistance and instructions.

The information contained herein is to our knowledge true and correct and presented in good faith. However, no warranty, guarantee, or freedom from patent infringement is implied or inferred. This information is offered solely for your consideration and verification.

Appendix B-3: Certificate of Analysis

CHR HANSEN

ABT-5
 Certificate of Analysis

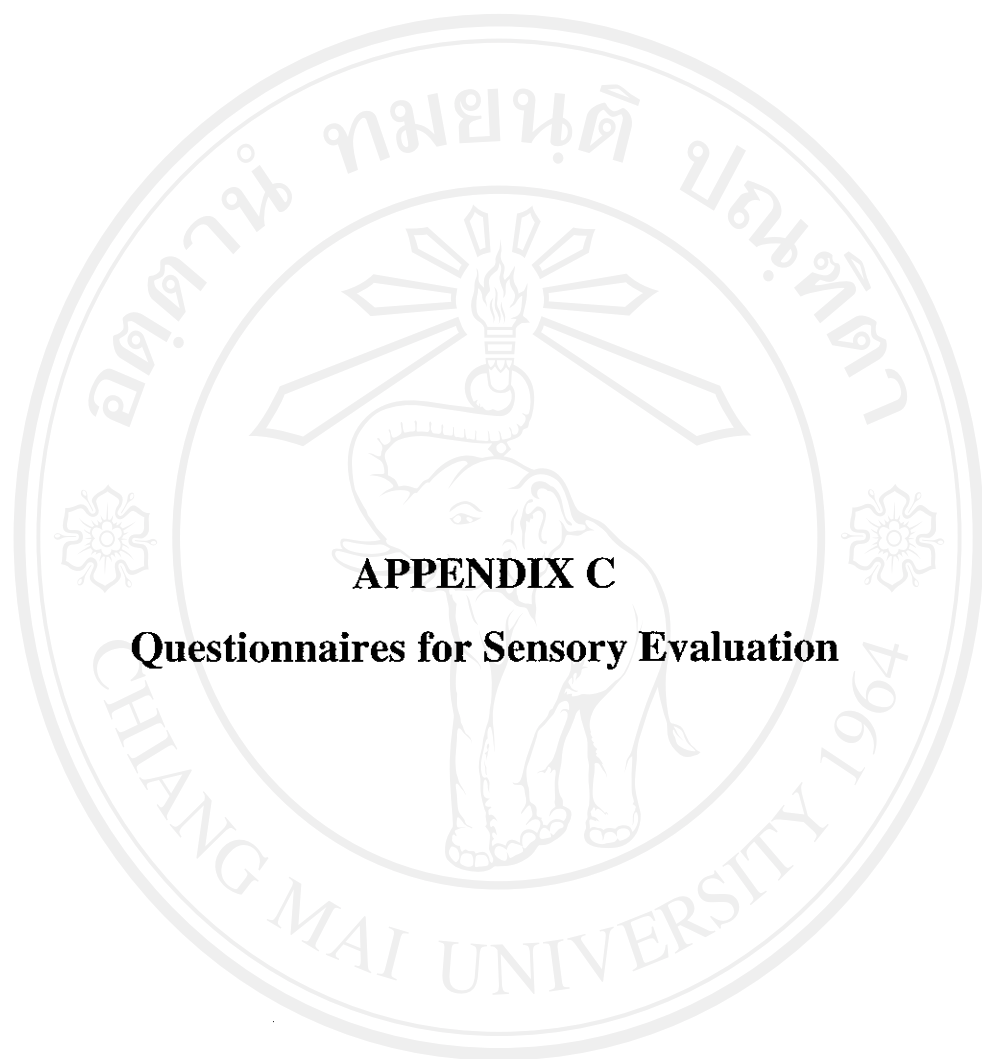
From: Freeze-dried DVS
 Item no: 100134
 Batch no: 2582200
 Best before Date: 12/2006

Performance	Result	Specification
pH 6h 40°C [pH]	4.6	4.4-4.7
Total cell count [cfu/g]	8.73E+10	> =5E+10

Purity	Result	Specification
Coliform [MPN/g]	< 1.0	< 10
Enterococci [cfu/g]	< 100	< 100
Mould [cfu/g]	< 10	< 10
Non lactic acid bacteria [cfu/g]	80	< 500
<i>Staphylococcus aureus</i> [cfu/g]	< 10	< 10
Yeast [cfu/g]	< 10	< 10
<i>Salmonella spp.</i> *	* See note below	Absent in 25 g
<i>Listeria monocytogenes</i> *	* See note below	Absent in 25 g

* Production is systematically tested on an ongoing basis – details can be supplied on request.

Conditions for activity analysis
 pH 6 h 43°C 500U / 2500 L



APPENDIX C

Questionnaires for Sensory Evaluation

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Appendix C-1: Product Profile Test

(To find out the sensory characteristics of green soya bean yoghurt ice cream and the ideal level for each characteristic, Chapter 3 section 3.5.2)

Name.....Date.....

Green soya bean yoghurt ice cream

Please rinse your mouth with water before starting. Observe and taste the sample. Please write down product characteristics, their scales and their units that you consider to be important for the product. Please give your opinion for each product characteristic with X and I symbols on the straight line.

I is an ideal level for the product characteristic or the characteristic level that you like to be.

X is the sensory characteristics level of the product sample.

Product definition

1. Appearance

.....|-----|

.....|-----|

.....|-----|

2. Flavor

.....|-----|

.....|-----|

.....|-----|

3. Texture

.....|-----|

.....|-----|

.....|-----|

4. Overall

|-----|

Suggestion

.....

.....

Thank you for your participation.

Appendix C-2: Sensory Test

(To find the ideal level for green soya bean yoghurt ice cream, Chapter 3 section 3.5.2)

Name.....Date.....

Green soya bean yoghurt ice cream

Please rinse your mouth with water before starting. Observe and taste the sample. Please give your opinion for each product characteristic with (×) and (I) symbols on the straight line.

I is an ideal level for the product characteristic or the characteristic level that you like to be.

× is the sensory characteristics level of the product sample.

Product characteristics

1. Green color

	Light	Dark
2. Smoothness

	Low	High
3. Yoghurt flavor

	Low	High
4. Green soya bean flavor

	Low	High
5. Mouthfeel

	Quick	Slow
6. Sweetness

	Low	High
7. Sourness

	Low	High
8. Overall

	Not accept	Accept

Suggestion

.....

Thank you for your participation.

Appendix C-3: Sensory Test

(To find the panelists opinion to develop green soya bean yoghurt ice cream, Chapter 3 section 3.5.3 and 3.5.4)

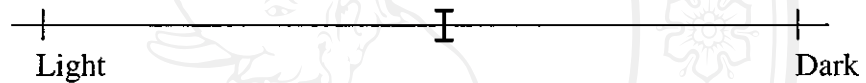
Name.....Date.....

Green soya bean yoghurt ice cream

Please rinse your mouth with water before starting. Observe and taste one by one sample in front of you. The fixed marks on the scale are the ideal level of the product characteristic. Please give your comparison opinion compared to the ideal value by marking (|) on the straight line below and wrote down the sample number on the mark for each product characteristic.

Product characteristics

1. Green color



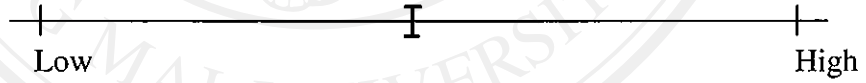
2. Smoothness



3. Yoghurt flavor



4. Green soya bean flavor



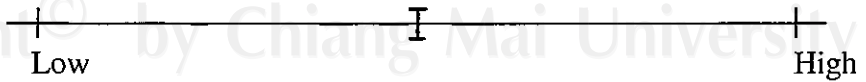
5. Mouthfeel



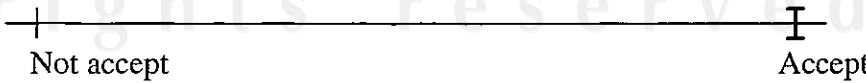
6. Sweetness



7. Sourness



8. Overall



Suggestion

.....

Thank you for your participation.

Appendix C-4: Sensory Test

(To find the panelists opinion during the shelf life study of green soya bean yoghurt ice cream, Chapter 3 section 3.5.5)

Name.....Date.....

Green soya bean yoghurt ice cream

Please rinse your mouth with water before starting. Observe and taste the sample. The fixed marks on the scale are the ideal level of the product characteristic. Please give your comparison opinion compared to the ideal value by marking (X) on the straight line below.

Product characteristics

1. Green color



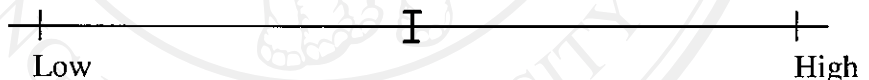
2. Smoothness



3. Yoghurt flavor



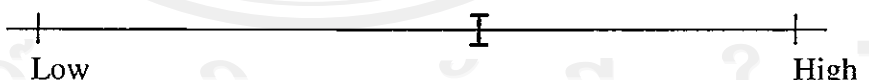
4. Green soya bean flavor



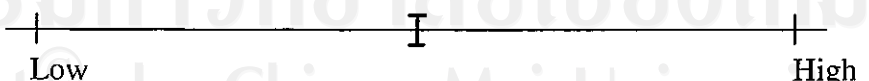
5. Mouthfeel



6. Sweetness



7. Sourness



8. Overall



Suggestion

.....

Thank you for your participation.



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Appendix D-1: Physical Analyses

1. Color (Hunter lab)

Color evaluation was performed on green soya bean, green soya bean milk and green soya bean yoghurt ice cream samples using a colorimeter, Minolta Chroma Meter Model CR-300 Series, Japan. The instrument was calibrated with a white tile. The Hunter L*, a*, and b* scales give a measurement of color in units of approximate visual uniformity throughout a liquid. L* value represented the lightness of color, a* value represented the greenness and redness and b* value represented the blueness and yellowness.

- L* value measures lightness and varies from 100 for perfect white to zero for black.

- a* value measures redness when positive (+) and greenness when negative (-) with maximum values of 60.

- b* value measures yellowness when positive (+) and blueness when negative (-) with maximum values of 60.

Each value represented a mean value of three determinations for each sample.

2. Overrun (Arbuckle, 1996)

Overrun measurement was taken per sample by comparing the weight of ice cream mix and ice cream in a fixed volume container. The overrun value (%) was calculated with a formula as followed:

$$\text{Overrun (\%)} = \frac{\text{weight of mixture} - \text{weight of ice cream} \times 100}{\text{weight of ice cream}}$$

3. Melting rate (A modified method from Lee and White, 1991)

A sample of ice cream that was cut from a block of ice cream had a weight of 100 ± 1 g. The ice cream sample (initially at -13°C) was placed on a wire screen (4 mesh) on the top of a beaker. The ice cream was let to be melted in a controlled temperature chamber at 25°C . The dripped volume was recorded for 1 h.

$$\text{Melting rate (per 100 g)} = \frac{\text{weight of dripped volume} \times 100}{\text{weight of ice cream}}$$

Appendix D-2: Chemical Analyses

1. **Fiber** (a modified method from an AOAC official method no.978.10.) (AOAC, 2000)

1 g of sample is extracted for its fat content with petroleum ether and transferred into a 600 ml beaker. Add 200 ml of 0.255 N sulphuric acid solutions into the beaker. Boil the solution and leave the solution boils exactly for 30 min. Prepare a funnel to filter the fiber (using a whatman paper no. 541 that is known accurately its weight). Flow boiling distilled water through the funnel to warm it. Then pour the acid solution that was boiled through the funnel and wash the solid that is collected on the funnel with boiled distilled water using vacuum. Wash the residue from the funnel into the beaker that is used before with 200 ml of 0.313 N sodium hydroxide solutions. Place the beaker on a heater and boil for 30 min. Afterwards, pour the base solution that was boiled through a funnel using a whatman paper no. 541 that is known accurately for its weight. Wash the solid on the funnel with boiled distilled water using vacuum. Transfer the residues into a crucible that is known accurately for its weight. Dry the crucible in a boiling-water bath and then take the crucible into a hot air oven at 100°C until the weight of the crucible is constant. Cool the crucible in an active desiccator and weight again. After that, take the crucible into an oven at 500°C for 3 h. Cool the crucible in an active desiccator and weight. Calculate the percentage of fiber in the sample.

$$\text{Fiber (\%)} = \frac{\text{weight of ash (g)} \times (100 - \% \text{H}_2\text{O} - \% \text{fat})}{\text{weight of sample (g)}}$$

2. Fat by a Rose-Gottlieb method (a modified method from an AOAC official method no. 905.02.) (AOAC, 2000)

Weigh 5 g of sample into a separatory funnel. Add 10 ml distilled water and 2 ml NH_4OH and mix thoroughly. Add 10 ml ethyl alcohol 95% and mix well. Next, add 25 ml diethyl ether that must be peroxide-free, and then close with a stopper, shake very vigorously for 1 min. After that, add 25 ml petroleum ether and repeat vigorous shaking for 1 min. Let the funnel stand for 30 min (the liquid is separated into two layers). Separate the upper liquid into a beaker that is dried and known accurately for its weight. Repeat the extraction of the remaining liquid in the separatory funnel twice, using 15 ml diethyl ether and 15 ml petroleum ether. The upper liquid that is extracted for 3 times is added together into the dried beaker. Then, the beaker is taken into a hot air oven at 100°C , and dried to a constant weight. Transfer to a desiccator, cool, and weight.

$$\text{Fat (\%)} = \frac{\text{weight of fat (g)} \times 100}{\text{weight of sample (g)}}$$

3. Fat by a Soxhlet extraction method (a modified method from an AOAC official method no. 948.16 and 991.36.) (AOAC, 2000)

Weigh 2 g of dry sample into a whatman paper no.4 and put in the paper with its content in an extraction thimber. Extract the sample with petroleum ether in a continuous extractor for 3 h. Fat is separated into a flask that is dried and known accurately for its weight. Distil off the petroleum ether until the volume in the flasks is 10-15 ml. Then, the flask is taken into a hot air oven at 100°C for 30 min. Transfer to a desiccator, cool, and weight.

$$\text{Fat (\%)} = \frac{\text{weight of fat (g)} \times 100}{\text{weight of sample (g)}}$$

4. Protein by a Kjeldahl method (a modified method from an AOAC official method no.991.20.) (AOAC, 2000)

Weigh 2 g of green soya bean yoghurt ice cream into a Kjeldahl digestion flask. Add 8 g of catalyst mixture (96% sodium sulphate and 4% Copper sulphate) and 20 ml sulphuric acid into the digestion flask. Place the flask in an incline position in a digestion machine. Start the digestion on heating low enough so that the sample does not foam up to the neck of the digestion flask. Next, increase the burner setting and boil until the sample is looked clear and then cool it to room temperature. Add methyl red/bromocresol green indicator into a boric solution that is used to collect distillate of the sample. Do distillation with the cool digestion flask. After the distillation is over, titrate the distillate with 0.05 M sulphuric acid.

Calculate the percentage of nitrogen in the sample (1 ml 0.1 N sulphuric acid equal to 0.0014 g nitrogen). The crude protein figure can be calculated using an appropriate factor of 6.38.

$$\text{Nitrogen (\%)} = \frac{(\text{ml of H}_2\text{SO}_4 - \text{ml of H}_2\text{SO}_4 \text{ blank}) \times \text{N. H}_2\text{SO}_4 \times 1.4007}{\text{weight of sample (g)}}$$

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.38$$

5. Ash (a modified method from an AOAC official Method no.945.46.) (AOAC, 2000)

Weigh 1 g of sample and place on a crucible that is known accurately for its weight. Heat the crucible on a steam bath. Afterwards, transfer the crucible into a hot air oven at 500°C until the sample becomes an ash. Cool the crucible in an active desiccator and weigh it again. Calculate the percentage of ash in the sample.

$$\text{Ash (\%)} = \frac{\text{weight of ash (g)} \times 100}{\text{weight of sample (g)}}$$

6. Total acidity by a titrimetric method (a modified method from an AOAC official method no 947.05.) (AOAC, 2000)

Weigh 10 g of sample into a 250 ml flask and dilute the sample with distilled water to 100 ml. Add 2-3 dropped phenolphthalein indicator and titrate against 0.1 N NaOH until the first persistent pink appears. Calculate the total acidity by following an equation below;

$$\text{Lactic acid (\%)} = (\text{ml of 0.1N NaOH} \times 100) \times 0.009$$

7. Moisture content (a modified method from an AOAC official Method no. 925.45.) (AOAC, 2000)

Weigh 2 g of sample into moisture can with a tight-fit cover, which is known accurately the weight. Place loosely the covered can in a hot air oven at 100°C. Dry the samples until the weight is constant (about 6 h). Press the cover tightly into the moisture can, then remove them from the oven, cool in an active dessicator and weight. Express the loss weight as a moisture content of the sample.

$$\text{Moisture content (\%)} = \frac{\text{weight of dry sample (g)} \times 100}{\text{weight of sample (g)}}$$

8. Carbohydrate (AOAC, 2000)

Carbohydrate (%) was calculated with a formula as followed:

$$\text{Carbohydrate (\%)} = 100 - (\% \text{ Moisture content} + \% \text{ Fat} + \% \text{ Protein} - \% \text{ Fiber} + \% \text{ Ash})$$

Appendix D-3: Microbiological Analyses

Media

1. Maximum Recovery Diluent: MRD

Typical composition (g/l):

Peptone	1.0
Sodium chloride	8.5

Preparation:

Dissolve 9.5 g of the mixture ingredient in 1 l of distilled water. Dispense into final containers and sterilize by autoclaving at 121°C for 15 min. The final pH was 7.0 ± 0.2 at 25°C.

2. Lauryl Tryptose Broth

Typical composition (g/l):

Tryptose	20.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen phosphate	2.75
Sodium chloride	5.0
Lactose	5.0
Sodium lauryl sulphate	0.1

Preparation:

Suspend 35.6 g of the mixture ingredient in 1 l of distilled water by heating in a boiling water bath and distribute 9 ml into test tubes each containing an inverted Durham tube. Sterilize by autoclaving at 121°C for 15 min.

3. Potato Dextrose Agar: PDA

Typical composition (g/l):

Potato infusion	4.0 (infusion from 200 g potatoes)
D (+) Glucose	20.0
Agar	15.0

Tartaric solution 10% (w/v):

Add 10 g of tartaric acid to 100 ml of distilled water. Sterilize by autoclaving at 121°C for 15 min.

Preparation:

Suspend 39 g of the mixture ingredient in 1 l of distilled water by heating in a boiling water bath and sterilize by autoclaving at 121°C for 15 min. Cool the medium to 50°C and aseptically add 0.5% sterile tartaric solution (10% w/v). Mix well and distribute into final containers. The final pH after add tartaric solution was 5.6 ± 0.2 at 25°C.

4. Plate Count Agar: PCA

Typical composition (g/l):

Peptone from casein	5.0
Yeast extract	2.5
D (+) Glucose	1.0
Agar	14.0

Preparation:

Suspend 22.5 g of the mixture ingredient in 1 l of distilled water by heating in a boiling water bath and sterilize by autoclaving at 121°C for 15 min. The final pH was 7.0 ± 0.2 at 25°C.

5. M17 Agar

Typical composition of M17 broth (g/l):

Tryptone	5.0
Soya peptone	5.0
Meat digest	5.0
Yeast extract	2.5
Ascorbic acid	0.5
Magnesium sulphate	0.25
Di-sodium-glycerophosphate	19.0

Lactose solution 10% (w/v):

Add 10 g of lactose to 100 ml of distilled water. Sterilize by autoclaving at 121°C for 15 minutes.

Preparation:

Add 37.25 g of broth mixture ingredient and 15 g of agar in 950 ml of distilled water by heating in a boiling water bath and sterilize by autoclaving at 121°C for 15 min. Cool the medium to 50°C and aseptically add 50 ml of sterile lactose solution (10% w/v) and 1% of sterile 1 N HCl. Mix well the solution and distribute into final containers. The final pH was 6.9 ± 0.2 at 25°C.

6. Homofermentative Heterofermentative Differential Agar: HHD

Typical composition (g/l) was prepared as described by Mc Donald *et al.* (1987):

D (-) Fructose	2.5
Potassium dihydrogen phosphate	2.5
Peptone from casein	10.0
Soytone	1.5
Casamino acids	3.0
Yeast extract	1.0
Tween 80	1.0
Agar	20.0

Dye solution:

Add 0.1 g of bromocresol green to 30 ml of 0.01 N NaOH. Sterilize by autoclaving at 121°C for 15 min.

Preparation:

Add all the ingredient in 1 l of distilled water by heating in a boiling water bath and adjust the pH to 7.0 ± 0.2 at 25°C by 1 N NaOH. Sterilize by autoclaving at 121°C for 15 min. Cool the medium to 50°C and aseptically add 2% of dye solution. Mix the medium well and distribute into final containers. The final pH was 7.0 ± 0.2 at 25°C.

Appendix D-4: US Standard Plate Count Guidance

(American Public Health Association, 1970)

Diluting samples**1. Work Area**

- a. Level plating bench not in direct sunlight.
- b. Sanitized immediately before start of plating.

2. Selecting Dilutions

- a. Plate three decimal dilutions per sample.
- b. Select dilutions to yield one plate with 30-300 colonies.

3. Identifying Plates

- a. Label each plate with sample identification and dilution.
- b. Arrange plates in order before preparation of dilutions.

4. Sample Agitation

- a. When appropriate, wipe top of unopened containers with sterile, ethyl alcohol-saturated cloth.
- b. Remove test portion within 3 min of sample agitation.

5. Sample Measurement, pipettes

- a. Use separate sterile pipettes for the initial transfers from each container.
Pipettes in a pipette container adjusted without touching the pipettes.
- b. Pipette tip not dragged over exposed exterior of pipettes in the container.
- c. Pipette not dragged across lip or neck of a sample container.
- d. Pipette not inserted more than 2.5 cm (1") below sample surface (foam avoided if possible).

- e. Draw test portion above pipette graduation mark and remove pipette from liquid. Can use a pipette aid, mouth pipetting not permitted.
- f. Adjust test volume to mark with lower side of pipette in contact with inside of sample container (above the sample surface).
- g. Drainage complete, excess liquid not adhering to pipette.
- h. Release test portion to a petri dish (tip in contact with plate, 45°angle) or dilution blank (with lower side of pipette in contact with neck of dilution blank, or dry area above buffer where appropriate) with column drain of 2-4 s.
- i. Blow out last drop of undiluted sample from the pipette using the pipette aid. Blow out away from the main part of sample in plate, do not make bubbles.
- j. Pipettes discarded into disinfectant, or if disposable into biohazard bags or containers to be sterilized.

6. Dilution Agitation

- a. Optionally, use an approved mechanical shaker for 15 s.
- b. Remove test portion within 3 min of dilution agitation.

7. Dilution Measurement, pipettes

- a. Use separate sterile pipettes for the initial transfers from each container. Pipettes in a pipette container adjusted without touching the pipettes.
- b. Pipette tip not dragged over exposed exterior of pipettes in the container.
- c. Pipette not dragged across lip or neck of dilution blank.
- d. Pipette not inserted more than 2.5 cm below dilution surface.
- e. Draw dilution portion above pipette graduation mark and remove pipette from liquid. Can use a pipette aid, mouth pipetting not permitted.
- f. Adjust dilution volume to mark with lower side of pipette in contact with inside of dilution blank neck.
- g. Drainage complete, excess liquid not adhering to pipette.
- h. Gently lift cover of a petri dish just high enough to insert the pipette.
- i. Hold the pipette at 45°angle to dish with tip touching dish (or dilution blank neck).

- j. Release dilution portion to dish (or dilution blank) with tip in contact with the bottom of the dish (or dilution blank neck or dry area above buffer where appropriate) with column drain of 2-4 s.
- k. Touch pipette tip once against dry spot on the dish bottom (or dilution blank neck). When measuring 0.1 ml, do not re-touch dry area.
- l. Pipettes discarded into disinfectant, or if disposable into biohazard bags or containers to be sterilized.

Plating

8. Plating

- a. Melt agar quickly in boiling water, flowing steam not under pressure, or microwave oven (use extreme caution when microwaving).
- b. Avoid prolonged exposure to high temperatures during and after melting.
- c. Do not melt more than will be used within 3 hours.
- d. Do not melt agar more than once.
- e. Promptly cool melted agar to $45 \pm 1^\circ\text{C}$. Record the temperature with other control information.
- f. Temperature control used for each test medium type:
 1. Contains medium identical to type being used.
 2. In container identical to type being used.
 3. Undergoes same heat treatment and cooling as test medium.
- g. Select number of samples in any series so that all will be plated within 20 min after diluting first sample.
- h. After depositing test portions, promptly pour 10-12 ml medium into each plate of series, or 15-20 ml for > 1 ml portion/plate or where agar weight loss is a problem that can not be corrected by other actions.
- i. Lift cover of a petri dish just high enough to pour medium.
- j. As each plate is poured thoroughly and evenly, mix medium and test portion in the petri dish. Multiple plates may be poured and mixed, however, plates may not be stacked prior to mixing.
- k. Allow to solidify within 10 min on level surface.

Controls

9. Controls

- a. Check sterility of dilution blanks, medium, petri dishes and pipettes used for each group of samples.

Counting Colonies

10. Counting Aids

- a. Count colonies with aid of magnification under uniform and properly controlled artificial illumination with a hand tally.

11. Recording Standard Plate Count

- a. After incubating plates, promptly count all colonies on selected plates.
- b. Where impossible to count at once, store plates at 0-4.4°C for not longer than 24 h (avoid as a routine practice).
- c. Record dilutions used and number of colonies on each plate counted.
- d. Record results of sterility and control tests.
- e. When possible, select spreader free plates with 30-300 colonies and count all colonies:
 1. Use higher magnification if necessary to distinguish colonies from foreign matter.
 2. Examine edge of plates for colonies.
- f. If consecutive plates yield 30-300 colonies, count all colonies on plate from both dilutions.
- g. Count chains from separate source as separate colonies.
- h. If there is no 30-300 colony plate, use plate having nearest to 30-300 colonies.
- i. If plates from all dilutions exceed 300 colonies, estimate counts as follow:
 1. Count colonies in portions representative of distribution and estimate total.
 2. Where there are < 10 colonies/sq cm, count colonies in 12 squares, selecting 6 consecutive squares horizontally across the plate and 6 consecutive squares at right angles.

3. When there are 10 or more colonies/sq cm, count 4 representative squares.
4. Multiply average number colonies/sq cm by area of plate in sq cm.
- j. If plates yield < 30 colonies each, record actual number in lowest dilution.
- k. If all plates from a sample show no colonies, record count as 0.

12. Personal Errors

- a. Avoid inaccurate counting due to carelessness, fatigue or impaired vision.
- b. Discover cause and correct if unable to duplicate your own counts on the same plate.

Reports

20. Computing and Reporting Counts

- a. Multiply number of colonies (or estimated number if necessary) by the reciprocal of the dilution.
- b. If consecutive dilutions yield 30-300 colonies, compute count using a formula below:

$$N = \sum C / [(1 \times n_1) + (0.1 \times n_2)] \times d$$

Where ; N = number of colonies per milliliter or gram

$\sum C$ = sum of all colonies on all plates counted

n_1 = number of plates in lower dilution counted

n_2 = number of plates in the next highest dilution counted

d = dilution from which the first counts were obtained

Example ; 1:100 = 244 colonies and 1:1,000 = 38 colonies

$$\begin{aligned} N &= (244 + 38) / [(1 \times 1) + (0.1 \times 1)] \times 0.01 \\ &= 282 / [1.1] \times 0.01 \\ &= 282 / 0.011 \\ &= 25,636 \end{aligned}$$

Appendix D-5: Gram's Staining Method (Harrigan, 1998)**1. Gram staining reagents****1.1 Crystal violet stain**

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

1.2 Decolourizer

95% ethanol	250 ml
Acetone	250 ml

1.3 Gram iodine solution

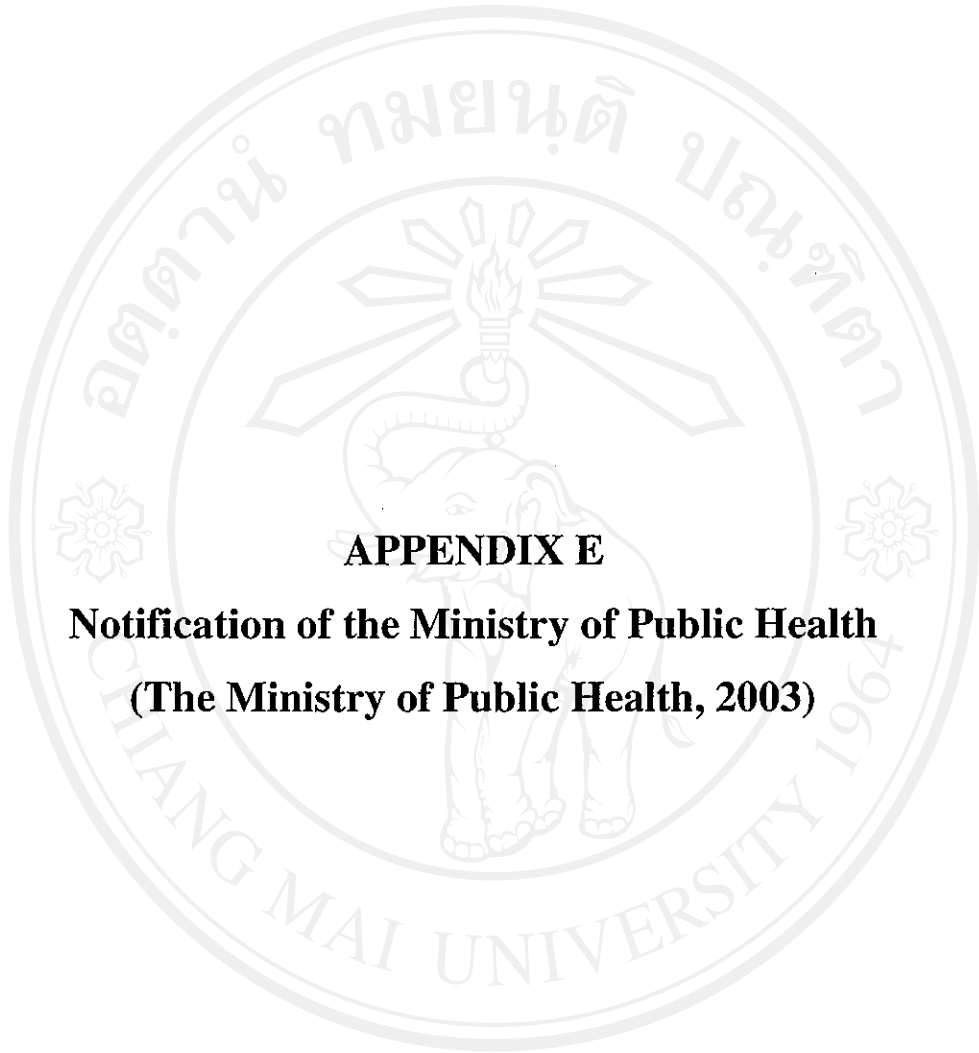
Iodine	1.0 g
Potassium iodide	2.0 g
Distilled water	300 ml

1.4 Safranin O solution

Safranin O	0.25 g
Distilled water	100 ml

2. Procedure

- 2.1 Prepare a heat-fixed smear from an culture.
- 2.2 Stain with crystal violet solution for 2 min. Take care that the entire smear is covered with the liquid at this and at all subsequent stages.
- 2.3 Holding the slide at an angle of 45°, wash the crystal violet solution off the smear. When all traces of the crystal violet solution have been removed, allow the iodine solution to act for 1 min.
- 2.4 Pour off the iodine, blot dry and holding the slide at an angle of 45°, wash the slide with a decolourizer until no more violet stain runs from the slide (only 5-15 s. in the case of well-prepared thin smears).
- 2.5 Rinse under the tap and stain with safranin O solution for 15 s.
- 2.6 Wash the slide well and blot dry.



APPENDIX E

Notification of the Ministry of Public Health

(The Ministry of Public Health, 2003)

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Notification of the Ministry of Public Health

(No. 222) B.E. 2544 (2001)

Re: Ice Cream.

It deems appropriate to amend the notification of the Ministry of public Health, Re: Ice Cream.

By the virtue of provisions of Section 5 and 6 (1) (2) (4) (5) (6) (7) and (10) of the Food Act B.E. 2522 (1979), in which contain provisions in relation to the restriction of Rights and Liberties of the Persons, in respect of which Section 29 and in conjunction with Section 35, Section 48 and Section 50 of the Constitution of the Kingdom of Thailand so permit by virtue of provisions of law; the Minister of Public Health hereby issues the notification as follows:

Clause 1. The following notifications shall be repealed:

(1) The notification of the Ministry of Public Health No. 33 B.E. 2522 (1979), Re: Prescribed ice cream to be specific controlled food and prescribed qualities or standards and production processes, dated 13th September B.E. 2522 (1979).

(2) The notification of the Ministry of Public Health No. 101 B.E. 2529 (1986), Re: Prescribed ice cream to be specific controlled food and prescribed qualities or standards and production processes (No. 2), dated 7th July B.E. 2529 (1986).

Clause 2. Ice cream is prescribed to be specific controlled food.

Clause 3. Ice cream as stipulated in Clause 2 shall be classified into 5 types:

(1) Milk ice cream shall be ice cream made from milk or milk products.

(2) Modified ice cream shall be ice cream, as stipulated in (1), made from all or in parts of other substituted fat to milk fat or ice cream which is made from other fat products which are not products from milk.

(3) Mixed ice cream shall be ice cream as stipulated in (1) or (2), as the case may be, which have fruits or other kind of foods content.

(4) Ice cream as stipulated in (1), (2), or (3) which are in liquid, dry, or powder forms.

(5) Edible ice shall be ice cream which is made from water and sugar or other kind of foods content.

Ice cream as stipulated above may also have flavouring agents and colouring agents as ingredients.

Clause 4. Every kind of ice cream, except ice cream as stipulated in 3(4), shall be respectively passed processes as follows:

(1) Being passed through heating by one of the following methods:

(1.1) Being heated up to the temperature of 68.5 degree Celsius and remaining at this temperature not less than 30 minutes or,

(1.2) Being heated up to the temperature of 80 degree Celsius and remaining at this temperature not less than 25 seconds and shall provide temperature meter with automatic recording and displaying true temperature or,

(1.3) Being heated up by any other methods which are approved by the Food and Drug Administration.

(2) Immediately being cool down to 4 degree Celsius and remaining at this temperature.

(3) Being spun, stirred, or mixed, as the case may be, and frozen at the temperature of not more than - 2.2 degree Celsius before being packed for sale and shall be kept at the temperature of not more than - 2.2 degree Celsius until the ice cream is being sold.

Clause 5. Ice cream is prescribed food to have qualities or standards as follows:

(1) Milk ice cream is prescribed to have milk fat content of not less than 5% by weight and contain milk solid non fat not less than 7.5% by weight.

(2) Modified ice cream is prescribed to have total fat of not less than 5% by weight.

(3) Mixed ice cream is prescribed to have standards as stipulated in (1) or (2), as the case may be, excluding total weight of fruits or other kind of ingredients.

(4) Edible ice and ice cream as stipulated in Clause 3(1) (2) or (3) shall be:

(4.1) Free of rancid odour.

(4.2) Artificial sweetener shall follow to Food Standard of Joint FAO/WHO Codex, Re: Food additives and the amended version and may be used in single or combination with sugar.

In cases where no standard is prescribed in the first phrase, the Food and Drug Administration shall prescribe according to the Food Committee.

(4.3) Free of preservatives.

(4.4) Total bacterial not more than 600,000 in 1 gm. of food.

(4.5) Escherichia coli shall not be detected in 0.01 gm. of food.

(4.6) Free of pathogenic microorganisms.

(4.7) Free of toxic substances released by microorganisms in quantity which may be hazardous to health.

(5) Liquid ice cream is prescribed to have qualities or standards as stipulated in (1) (2) or (3), as the case may be, and qualities or standards as stipulated in (4) shall be accomplished as well.

Clause 6. Ice cream in dry or powder form is prescribed to have qualities or standards as follows:

(1) Free of rancid odour.

(2) Odour inherent of that specific characteristics of such ice cream.

(3) Shall not appear to be lump which is changed from its characteristics made up.

(4) Artificial sweetener shall follow to Food Standard of Joint FAO/WHO Codex, Re: Food additives and the amended version and may be used in single or combination with sugar.

In cases where no standard is prescribed in the first phrase, the Food and Drug Administration shall prescribe according to the Food Committee.

(5) Free of preservatives.

(6) Moisture content not more than 5% by weight.

(7) Total bacterial not more than 100,000 in 1 gm. of food.

(8) Free of pathogenic microorganisms.

(9) Free of toxic substances released by microorganisms in quantity which may be hazardous to health.

Clause 7. Usage of food additives shall follow to the notification of the Ministry of public Health, Re: Food additives.

Clause 8. Ice cream producers or importers for sale shall follow to the notification of the Ministry of Public Health, Re: Production processes, production equipments and foods storages.

Clause 9. For ice cream packagings used shall follow to the notification of the Ministry of public Health, Re: Containers.

Clause 10. Labels of ice cream shall follow to the notification of the Ministry of Public Health, Re: Labels.

Clause 11. This notification:

(1) Shall not affect to Food Registration, which are issued according to the notification of the Ministry of Public Health No. 33 B.E. 2522 (1979), Re: Prescribed ice cream to be specific controlled foods and prescribed qualities or standards and production processes, dated 13 September B.E. 2522 (1979); the amendment of the notification of the Ministry of Public Health No. 101 B.E. 2529 (1986), Re: Prescribed ice cream to be specific controlled foods and prescribed qualities or standards and production processes (No. 2), dated 7 July B.E. 2529 (1986), prior to the come into force date of this notification, shall be further come into force.

(2) Food Labelling, which are issued to follow the notification of the Ministry of Public Health No. 68 B.E. 2525 (1982), Re: Labels, dated 29 April B.E. 2525 (1982); the amendment of the notification of the Ministry of Public Health No. 95 B.E. 2528 (1985), Re: Labels (No. 2), dated 30 September B.E. 2528 (1985); and the related notifications prior to this notification, shall be valid for 2 years as from the come into force date of this notification.

Clause 12. Ice cream producers or importers, whose permits issued prior to this notification, shall apply for food serial number within one year as from the come into force date of this notification. After applying for food serial number, the ice cream producers or importers shall be abated from the stipulation in Clause 8 for a period of 2 years after this notification come into force. As a result, the remaining labels shall be allowed to be used until last but not exceeding to 2 years after this notification come into force.

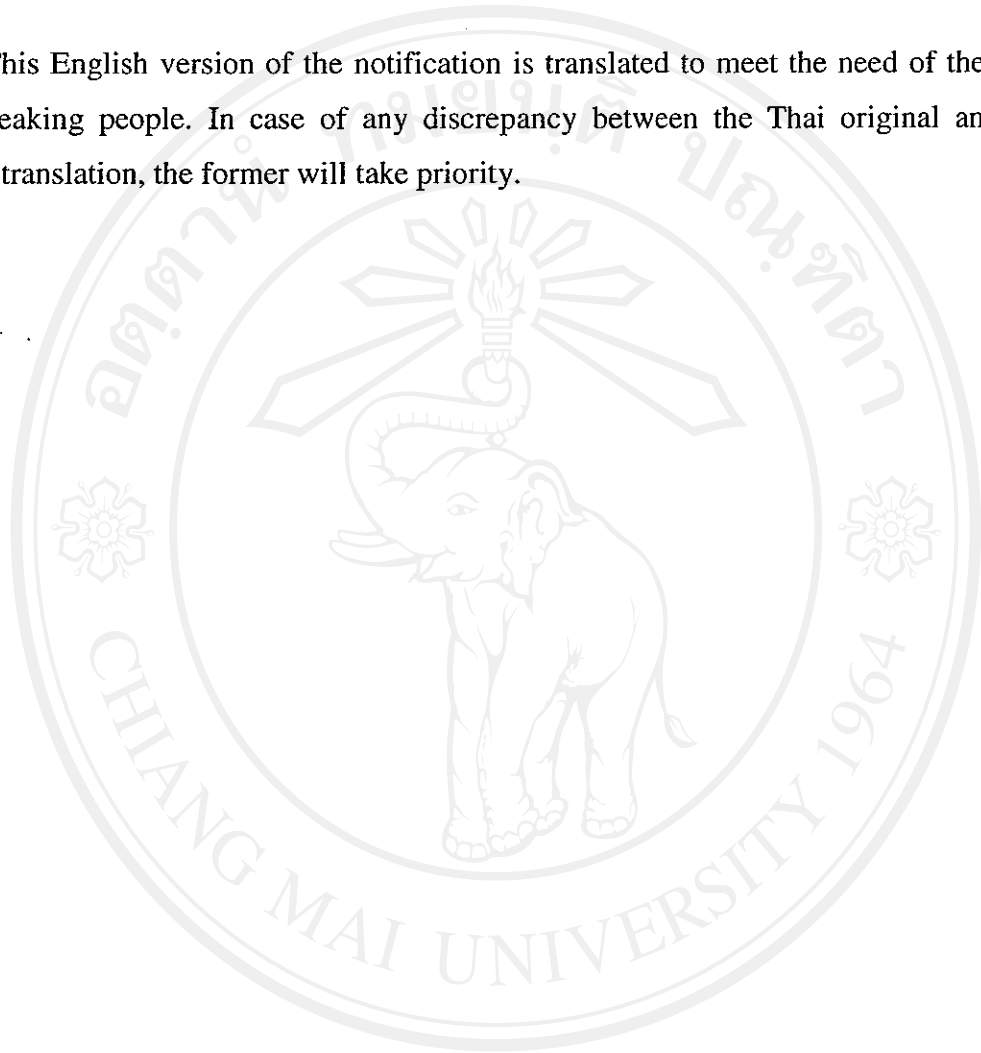
Clause 13. This notification shall come into force as from 24th July B.E. 2544 (2001).

Notified on 30th May 2002.

Signed Sudarat Keyurabhun
(Mrs. Sudarat Keyurabhun)
Minister of Public Health

(Published in the Government Gazette Vol. 118, Special Part 70 Ngor, dated 26th July 2001)

Note: This English version of the notification is translated to meet the need of the non-Thai speaking people. In case of any discrepancy between the Thai original and the English translation, the former will take priority.



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Notification of the Ministry of Public Health

(No. 257) B.E. 2545 (2002)

Re: Ice Cream (No. 2).

It deems appropriate to amend the notification of the Ministry of Public Health, Re: Ice cream.

By the virtue of provisions of Section 5 and 6(10) of the Food Act B.E. 2522 (1979), in which contain provisions in relation to the restriction of Rights and Liberties of the Persons, in respect of which Section 29 and in conjunction with Section 35, Section 48 and Section 50 of the Constitution of the Kingdom of Thailand so permit by virtue of provisions of law; the Minister of Public Health hereby issues the notification as follows:

Clause 1. The expressions in Clause 10 of the notification of the Ministry of Public Health (No. 222) B.E. 2544 (2001), Re: Ice cream, dated 24th July B.E. 2544 (2001), shall be repealed. The following expressions shall be used instead:

Clause 10 Labels of ice cream shall follow to the notification of the Ministry of Public Health Re: Labels.

In cases where labels are attached, sticked, or expressed on serving size of ice cream containers, the expression as stipulated in 3(11) of the notification of the Ministry of Public Health (No. 194) B.E. 2543 (2000), Re: Labels, dated 19th September B.E. 2543 (2000), shall be either expressed on the labels or on individual packages of ice cream containers.

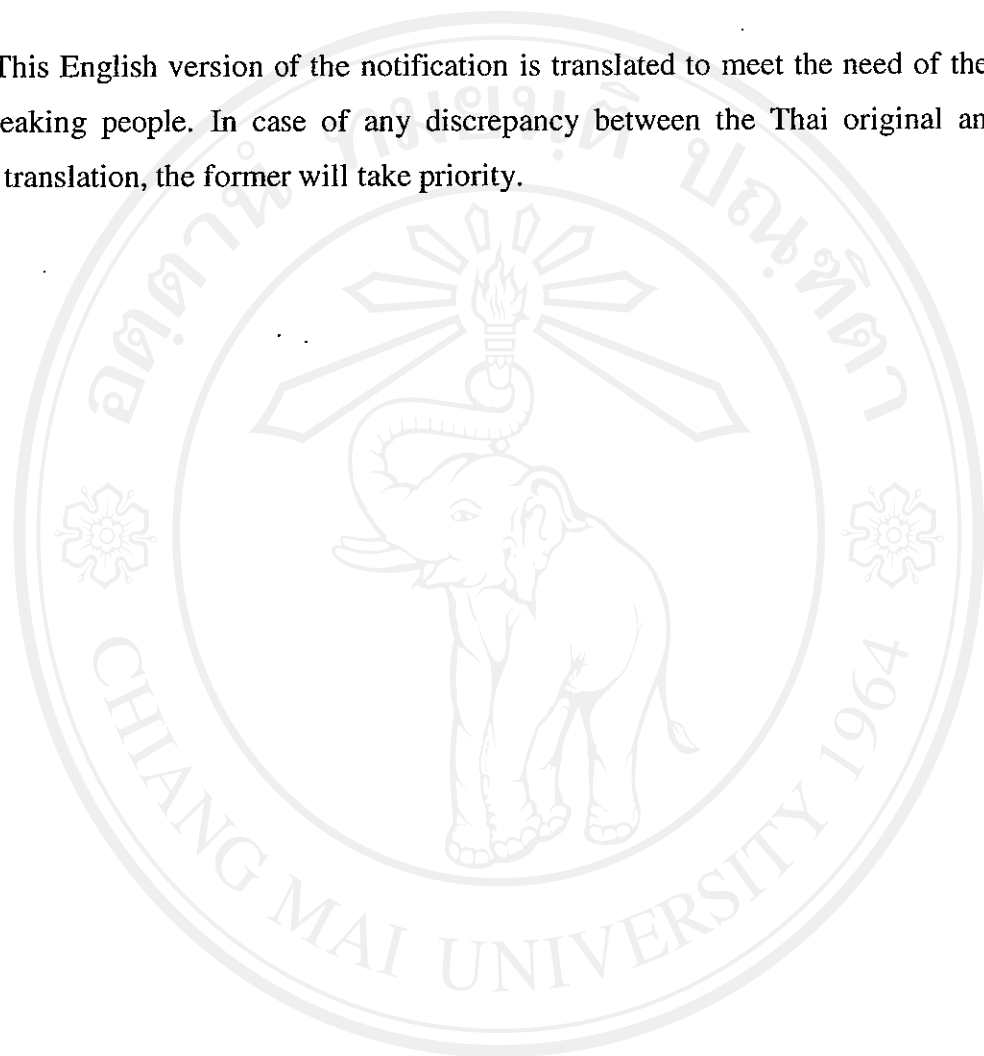
Clause 2. This notification shall come into force as from the day following date of its publication in the Government Gazette.

Notified on 30th May 2002.

Signed Sudarat Keyurabhun
(Mrs. Sudarat Keyurabhun)
Minister of Public Health

(Published in the Government Gazette Vol. 119, Special Part 54 Ngor, dated 18th June 2002)

Note: This English version of the notification is translated to meet the need of the non-Thai speaking people. In case of any discrepancy between the Thai original and the English translation, the former will take priority.



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