

## CHAPTER III

## MATERIALS AND METHODS

3.1 Materials3.1.1 Samples

Samples of raw and pasteurized milk of the same lot were collected from five dairies, three located in Bangkok and two in up-countries. They were

- 1) Foremost Dairy Plant (Bangkok)
- 2) Kasetsart University Dairy Plant (Bangkok)
- 3) Dairy Farm Promotion Organization of Thailand (Saraburi)
- 4) Nong-Po Cooperative (Rachaburi) and
- 5) Chitladda Royal Palace Dairy Plant (Bangkok)

See more detail in Table 1.4 P. 6a

Samples were collected from each dairy mentioned above three times in a period of 6 months. Each collection consisted of three bottles of 100 ml precooled raw milk, sampled under aseptic precautions in sterile glass bottles with double covers (aluminium foil with cap), and 40 packs of 225 ml (or  $\frac{1}{2}$  pint) pasteurized milk. The samples in Bangkok were picked up at the sites after processing. The samples from up-country dairies were delivered to their distribution centers in Bangkok by insulated truck and so, were collected there on the following day. All samples were carried to the Food Technology Department, Chulalongkorn University by keeping in an insulated box with ice packs to maintain the temperature at about 4°C. The analysis of raw milk was conducted immediately after arrival. Samples of pasteurized milk was kept in the

refrigerator with temperature controlled at  $5 \pm 1.0^\circ\text{C}$ ,  $7 \pm 1.0^\circ\text{C}$ ,  $10 \pm 1.0^\circ\text{C}$ ,  $15 \pm 1.0^\circ\text{C}$  and  $20 \pm 1.0^\circ\text{C}$  and analysed at the days shown in Table 3.1

Table 3.1 Schedule of sampling

Storage temperature ( $^\circ\text{C}$ )	Sampling days ( $\checkmark$ )																		Total samples			
	0	1	2	3	4	5	6	7	8	9	10	11	13	15	16	17	20	23		27	30	
5	$\checkmark$			$\checkmark$			$\checkmark$				$\checkmark$			$\checkmark$		$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	10
7	$\checkmark$			$\checkmark$		$\checkmark$		$\checkmark$		$\checkmark$		$\checkmark$		$\checkmark$		$\checkmark$						9
10	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$											12
15	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$																6
20	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$																		4

For each sample, it was divided into 2 sterile-glass bottles. One was analyzed for microbiological qualities, enzymatic properties and titrated for acidity at Food Technology Department Laboratory. The other was kept in insulated box with ice packs to maintain the temperature at about  $4^\circ\text{C}$  and brought to Veterinary Public Health Section, Department of Livestocks Development for analysis of chemical composition and somatic cell counts.

### 3.1.2 Media and reagents

- Plate count agar (PCA)
- Violet red bile agar (VRB)
- Bacto lactobacilli MRS agar (MRS)
- Phosphate buffer solution, pH 7
- 0.1 N sodium hydroxide solution

- 0.5% phenolphthalein solution
- dye stuff, ethidium bromide

### 3.1.3 Equipments

- Cooled incubator, 7°C
- Incubator, 32°C
- Refrigerators with different temperature controlled at 5, 7, 10, 15 and 20°C
- Milko Scan 104
- Fossomatic 90

## 3.2 Methods of analysis

### 3.2.1 Microbiological analysis (Appendix A)

- a. Standard plate count (40), expressed as log cfu/ml milk.
- b. Psychrotrophic count (40), expressed as log cfu/ml milk.
- c. Coliform count (40), expressed as log cfu/ml milk.
- d. Lactic acid bacterial count (21), expressed as log cfu/ml milk.

### 3.2.2 Chemical analysis(Appendix B)

- a. Titratable acidity (41, 42), expressed as percentage of lactic acid in milk
- b. Compositional analysis (fat, protein, lactose, water, total solid and solid not fat) by using Milko Scan 104 (43), expressed as percentage of each composition in milk.
- c. Somatic cell count by using Fossomatic 90(44), expressed as number of cells per ml milk.

### 3.2.3 Organoleptic analysis (Appendix C)

Using 5 panelists both lecturers and graduate students in Food Technology Department were evaluate the quality and acceptability of milk by scoring method. The panel was selected by their reproducibility and accuracy in testing of milk. The scores are color (1-3), odor (1-7), flavor (1-7), mouthfeel (1-3) and overall quality (1-9). The highest score indicated the best or extremely like; the lowest score indicated the worst of extremely dislike.

From the questionnaire (see appendix C), score used for each item was different. It was scaled by the sequence of principal. The most important item which was overall quality with the widest range of score and was used as the criteria for organoleptic quality.

### 3.2.4 Enzymatic analysis (Appendix D)

- Protease (44, 45)

- Lipase (44, 45)

### 3.3 Statistical Analysis (46, 47, 48, 49, 50)

The results were analysed statistically by using randomized complete block design to determine whether there was a difference of microbiological, chemical and organoleptical analysis among dairies in raw and pasteurized milk and the difference of chemical property at various storage temperatures and dairies. In addition, the linear and multilinear relationships between microbiological property and time of storage and between microbiological property were also determined.

The factorial with complete block design was also used to study the effect of storage temperatures and different dairies on shelf-life of pasteurized milk, then, using Duncan's Multiple Range Test to test the difference of the mean values. Samples of calculation were shown in Appendix E.