

**A STUDY OF THE METHOD FOR DETECTION OF ADDED  
MILK POWDER IN MILK AND PROCESSED MILK**



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entitled

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MILK POWDER IN MILK AND PROCESSED MILK**



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**A STUDY OF THE METHOD FOR DETECTION OF ADDED MILK POWDER IN MILK AND PROCESSED MILK****BENJAPORN NARINRAK 4536516 SCBT/M****M.Sc. (BIOTECHNOLOGY)****THESIS ADVISORS: PAIROJ LUANGPITUKSA, Ph.D., SAIYAVIT VARAVINIT, Ph.D., SUCHAT UDOMSOPAGIT, Ph.D.****ABSTRACT**

Currently, fresh milk products available in the market have been produced from pure fresh milk and raw milk combined with reconstituted milk. Due to the price of locally produced raw milk in Thailand, it is usually higher than the cost of imported milk powder. Some manufacturers want to reduce the cost of production so they combining reconstituted milk with raw milk during the production. This takes advantage of the consumer. Therefore, the development of the method for the detection of added milk powder in fresh milk is needed by the government in order to investigate whether the fresh milk available in the market is produced from only pure fresh milk as indicated on the label. This study was based on some changes in milk constituents including deoxyketone (lactulosyllysine) determination, biochemical analysis, ratios of milk composition, and milk fat particle size analysis. Results indicate that the ratios of milk composition can used as an index to differentiate between fresh milk and fresh milk that has been combined with reconstituted milk. This can be applied to the detection in market fresh milk (pasteurized and UHT milk). Deoxyketone determination and milk fat particle size analysis can differentiate between raw milk and raw milk combining with reconstituted milk. Biochemical analysis showed different values but not clear when compared with the ratios of milk composition. Therefore, biochemical analysis could not differentiate between fresh milk and fresh milk combined with reconstituted milk.

**KEY WORDS: DEOXYKETONE/ FRESH MILK/ LACTULOSYLLYSINE/  
MILK COMPOSITION / MILK POWDER / PARTICLE SIZE**

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บทคัดย่อ

ในปัจจุบันนี้ผลิตภัณฑ์นมสดที่จำหน่ายตามท้องตลาด มีทั้งที่ผลิตโดยใช้วัตถุดิบซึ่งมาจากนํ้านมดิบโดยตรงและผลิตโดยใช้วิธีการผสมระหว่างนํ้านมดิบและนมผงที่นำมาละลายนํ้า ทั้งนี้เนื่องจากต้นทุนในการผลิตนํ้านมดิบในประเทศมีราคาสูง จึงส่งผลให้นํ้านมดิบมีราคาสูงกว่านมผงที่นำเข้ามาจากต่างประเทศ ผู้ผลิตบางรายต้องการลดต้นทุนในการผลิตจึงผสมนมผงลงในนํ้านมดิบในระหว่างการผลิตซึ่งถือเป็นการเอาเปรียบผู้บริโภค ดังนั้นการพัฒนาวิธีการตรวจสอบการผสมนมผงลงในนมสดจึงมีความจำเป็นสำหรับหน่วยงานของรัฐ ในการตรวจสอบนมสดที่จำหน่ายตามท้องตลาดว่าผลิตมาจากนมสด 100% ตามที่ระบุไว้ในฉลากหรือไม่ ในศึกษานี้อาศัยการเปลี่ยนแปลงคุณสมบัติบางอย่างของส่วนประกอบนํ้านม ได้แก่ การตรวจหาปริมาณ deoxyketone หรือ lactulosyllisine, การตรวจหาส่วนประกอบทางชีวเคมีของนํ้านม, การวิเคราะห์อัตราส่วนของส่วนประกอบนํ้านมและการตรวจหา milk fat particle size จากการศึกษาพบว่า การวิเคราะห์อัตราส่วนของส่วนประกอบนํ้านม สามารถนำมาใช้เป็นดัชนีสำหรับจำแนกความแตกต่างระหว่างนมสดและนมสดที่มีการผสมนมผงได้อย่างชัดเจน และสามารถนำไปตรวจสอบนมสดที่จำหน่ายในท้องตลาดได้ ส่วนวิธีการตรวจหาปริมาณ deoxyketone และการตรวจหา milk fat particle size สามารถจำแนกความแตกต่างระหว่างนํ้านมดิบและนํ้านมดิบที่ผสมนมผงได้ สำหรับวิธีการตรวจหาส่วนประกอบทางชีวเคมีของนํ้ามนั้น ค่าที่ได้แตกต่างกันแต่ไม่ชัดเจนเมื่อเปรียบเทียบกับวิธีการวิเคราะห์อัตราส่วนของส่วนประกอบนํ้านม ทำให้ไม่สามารถแยกแยะความแตกต่างระหว่างนมสดและนมสดที่ผสมนมผงได้

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## ABBREVIATION



|      |                     |
|------|---------------------|
| °C   | Degree Celsius      |
| etc. | Et cetera           |
| e.g. | Exempli gratia      |
| g    | Grams               |
| i.e. | Id est              |
| kg   | Kilograms           |
| L    | Liter               |
| mg   | Milligrams          |
| ml   | Milliliters         |
| mM   | Millimolar          |
| nm   | Nanometers          |
| µm   | Micrometers         |
| RM   | Raw milk            |
| SMP  | Skimmed milk powder |
| WMP  | Whole milk powder   |

## CHAPTER I

### INTRODUCTION

Thailand is an agricultural country in which many people work on the land (Jedsadabundit P, 1998). Raw milk production is one of the important agriculture occupations in Thailand. Since the 1980s, local milk production has been increasing rapidly. As might be expected, not every region of Thailand is suitable for commercial milk production, and an indication of this variability is shown in Table 1. The reasons for these differences are primarily ecological, because whereas forage feeding is available all the year round in Ratchaburi Province, Khon Kaen is located in a much drier area. Other serious problems that often occur on dairy farms are low fertility and low conception rates, a high incidence of mastitis and low milk yields; the latter mainly result from a shortage of quality roughage, such as silage and hay, to provide the animals with sufficient feed, especially during the dry season (Kiyothong K, 1998).

Table 1 The 10 provinces in Thailand that had the highest numbers of dairy cows in 1995; all figures as the totals for each group.

| Province           | Dairy cows | Milking cows | Farms | Milk produced<br>(kg/day) |
|--------------------|------------|--------------|-------|---------------------------|
| Ratchaburi         | 55,186     | 22,465       | 5,036 | 180,017                   |
| Saraburi           | 30,899     | 20,480       | 2,221 | 230,434                   |
| Nakhon Ratchasima  | 28,737     | 15,955       | 1,111 | 98,731                    |
| Lopburi            | 28,219     | 13,677       | 1,600 | 87,721                    |
| Prachuap khirikhan | 13,104     | 7,196        | 975   | 53,990                    |
| Nakhon Pathom      | 11,039     | 5,482        | 622   | 32,587                    |
| Chiang Mai         | 10,673     | 5,935        | 1,757 | 53,990                    |
| Phetchaburi        | 9,812      | 5,448        | 936   | 28,496                    |
| Khon Kaen          | 7,457      | 2,038        | 394   | 15,117                    |
| Sa Kaew            | 6,081      | 3,329        | 297   | 25,181                    |

Source: Planning Division, Department of Livestock Development, 2000

In Thailand, ready to drink milk production use local raw milk for main material but amount of raw milk not enough for produced ready to drink milk. Therefore, some ready to drink milk are made from milk powder import from a foreign country. From Table 2 indicated that raw milk produced in Thailand insufficient for ready to drink milk production so skimmed milk powder was came in some ready to drink milk industry (skimmed milk powder 1kg. was recombined to ready to drink milk about 10kg.).

Table 2 Raw milk production and ready to drink milk production in 1992-1999.

| Year | Raw milk production (tonnes) | Ready to drink milk production (tonnes) | Skimmed milk powder for produced ready to drink milk (tonnes) |
|------|------------------------------|---|---|
| 1992 | 227,784                      | 293,669                                 | 6,580   |
| 1993 | 293,255                      | 352,240                                 | 5,890   |
| 1994 | 326,381                      | 413,848                                 | 8,740   |
| 1995 | 350,196                      | 527,467                                 | 17,700  |
| 1996 | 380,622                      | 607,687                                 | 22,700  |
| 1997 | 385,477                      | 608,883                                 | 22,340  |
| 1998 | 429,000                      | 556,140                                 | 12,700  |
| 1999 | 444,000                      | 589,480                                 | 14,500  |

Source: 1) Office of Agricultural Economics

- Raw milk production in 1992-1996
- Ready to drink milk production in 1992-1995

2) Department of Livestock Development

- Raw milk production in 1997-1998
- Ready to drink milk production in 1996-1998
- Year 1999 was estimated

According to the Notification of the Ministry of Public Health No.265 (B.E. 2545), fresh milk refers to milk obtained from a cow and is required to be free from colostrums, infectious disease, antibiotic, insecticide residues and no preservative. It

must contain not less than 2.8% by weight of milk protein. Fresh milk can be divided into three groups as follows:

1. Whole milk is defined as fresh milk from a cow with nothing removed or added. It contains at least 3.2% by weight of milk fat and at least 8.25% by weight of solid-not-fat.

2. Low-fat milk or partially skimmed milk contains 0.1% to 3.2% by weight of milk fat while most common products contain 2% milk fat. Low-fat milk must contain not less than 8.5% by weight of solid-not-fat.

3. Skimmed milk refers to milk that most of fat removed. It contains not less than 0.1% by weight of milk fat and at least 8.8% by weight of solid-not-fat.

Reconstituted milk is the liquid milk obtained from the addition of water to skimmed milk powder or whole milk powder (Alfa-laval, 1995). At present, there are three types of raw materials used for production of fresh milk including raw milk, raw milk combined with whole milk powder, and raw milk combined with skimmed milk powder (Hankunakul, 1999). The price of locally produced fresh milk in Asia is usually higher than the cost of imported milk powder (FFTC, 2001). To reduce the cost of production, some manufacturers produce fresh milk from milk powder or combination of raw milk and milk powder (Panaphon, 1999). However, manufacturers have to indicate the type of raw material used on labels of product. Nevertheless some manufacturers indicate that the raw material is raw milk although their milk products are produced from milk powder or combination of raw milk and milk powder (Hankunakul, 1999).

At present there are two factors that can be used to indicate added reconstituted milk, i.e. furosine and lactulose.

Furosine is a product of maillard reaction and can be obtained by acid hydrolysis of heated milk or milk product (Fennema, 1996). Addition of reconstituted milk during the production of consumption milk leads to abnormally high furosine value for pasteurized milk and to abnormally high ratio of furosine to lactulose for UHT milk (Leen, et al, 2000). Furosine can be determined by a HPLC method (Roland and Jan, 1996).

Lactulose, the formation of lactulose in heated milk due to the alkaline isomerization of lactose catalyzed by the free amino group of casein (Jan, et al, 1996). There are several analytical methods for detection of lactulose mainly, i.e. gas chromatography, enzymatic method based on spectrophotometric detection or amperometric detection (Amine, et al, 2000).

In order to develop the new methods for determination of pure fresh milk or it produced by combined with reconstituted milk, we focus in some constituents of milk that can be used to differentiate between fresh milk and milk combined with milk powder. In this study we investigated four factors including deoxyketone (Lactulosyllysine) determination, biochemical analysis, ratios of milk composition, and milk fat particle size analysis.

The purposes of this study were:

1. To evaluate the factors, that can differentiate between fresh milk and fresh milk combined with milk powder
2. To develop the method for detection of added milk powder into fresh milk
3. To develop appropriate method for detection of the presence of milk powder added to market fresh milk

## CHAPTER II

### LITERATURE REVIEW

Milk is a normal product of mammary gland secretion. It is composed of water, carbohydrate (lactose), fat, protein, minerals and vitamins. It is a complex, nutritious product that contains more than 100 substances that are either in solution, suspension or emulsion in water (Table 3). For example

- Casein, the major protein of milk, is dispersed as a great number of solid particles so tiny that they do not settle, but remain in suspension. These particles are called micelles. The dispersion of the micelles in milk is referred to as a colloidal suspension.

- Fat and fat soluble vitamins in the milk are in the form of an emulsion; a suspension of small liquid globules that does not mix with the water in milk.

- Lactose (milk sugar), some proteins (whey protein), mineral salts and other substances are soluble. They are entirely dissolved in the water in milk.

Casein micelles and fat globules play a major role on physical characteristics of milk and affect taste and flavor to dairy products such as butter, cheese, yoghurt, etc. The composition of milk varies considerably with the breed of cow, stage of lactation, feed, season of the year, and many other factors. However, some relationships between constituents are very stable and can be used to indicate whether any tampering with the milk composition has occurred. For example, milk of normal composition has a specific gravity in a range of 1.023 to 1.040 (at 20 °C) and a freezing point that varies from -0.518 to -0.534 °C. Any alteration, by addition of water for example, can be easily identified because these characteristics of milk will no longer be in the normal range. Milk is a highly perishable product that should be cooled to about 4 °C as soon as possible after collection. Extremes of temperature, acidity (pH) or contamination by microorganisms can rapidly decrease its quality.

Table 3 Nutritional composition of milk

| NUTRITIONAL COMPOSITION OF MILK<br>PER 100ML |                                   |                      |                                 |                        |
|--|-----------------------------------|----------------------|---------------------------------|------------------------|
| NUTRIENT                                     | Pasteurised<br>Channel<br>Islands | Pasteurised<br>Whole | Pasteurised<br>Semi-<br>Skimmed | Pasteurised<br>Skimmed |
| <b>Energy</b> , kcal                         | 80                                | 68                   | 48                              | 34                     |
| kJ   | 336                               | 284                  | 202                             | 145                    |
| <b>Protein</b> , g                           | 3.7                               | 3.3                  | 3.4                             | 3.4                    |
| <b>Carbohydrate</b> , g                      | 4.9                               | 4.9                  | 5.2                             | 5.2                    |
| of which sugars, g                           | 4.9                               | 4.9                  | 5.2                             | 5.2                    |
| <b>Fat</b> , g                               | 5.2                               | 4.0                  | 1.7                             | 0.1                    |
| of which saturates, g                        | 3.4                               | 2.5                  | 1.0                             | 0.1                    |
| monounsaturates, g                           | 1.3                               | 1.1                  | 0.5                             | Trace                  |
| polyunsaturates, g                           | 0.1                               | 0.1                  | Trace                           | Trace                  |
| <b>Cholesterol</b> , mg                      | 16                                | 14                   | 7                               | 2                      |
| <b>Sodium</b> , mg                           | 56                                | 57                   | 57                              | 57                     |
| <b>Water</b> , g                             | 88.9                              | 90.5                 | 92.9                            | 94.4                   |
| <b>Vitamin A</b> , µg                        | 60*                               | 58*                  | 23*                             | 1*                     |
| <b>Thiamin</b> , mg                          | 0.04†                             | 0.04†                | 0.04†                           | 0.04†                  |
| <b>Riboflavin</b> , mg                       | 0.20*                             | 0.18*                | 0.19*                           | 0.19*                  |
| <b>Niacin</b> , mg                           | 0.9†                              | 0.8†                 | 0.9†                            | 0.9†                   |
| <b>Vitamin B<sub>6</sub></b> , mg            | 0.06†                             | 0.06†                | 0.06†                           | 0.06†                  |
| <b>Vitamin B<sub>12</sub></b> , µg           | 0.4†                              | 0.4†                 | 0.4†                            | 0.4†                   |
| <b>Vitamin C</b> , mg                        | 1†                                | 1†                   | 1†                              | 1†                     |
| <b>Vitamin D</b> , µg                        | 0.03                              | 0.03                 | 0.01                            | Trace                  |
| <b>Vitamin E</b> , mg                        | 0.11                              | 0.09                 | 0.03                            | Trace                  |
| <b>Folate</b> , µg                           | 6†                                | 6†                   | 6†                              | 6†                     |
| <b>Pantothenate</b> , mg                     | 0.37                              | 0.36                 | 0.33                            | 0.33                   |
| <b>Biotin</b> , µg                           | 2.0                               | 2.0                  | 2.1                             | 2.0                    |
| <b>Calcium</b> , mg                          | 134                               | 119                  | 124                             | 124                    |
| <b>Chloride</b> , mg                         | 103                               | 103                  | 103                             | 104                    |
| <b>Copper</b> , mg                           | Trace                             | Trace                | Trace                           | Trace                  |
| <b>Iodine</b> , µg                           | N                                 | 15                   | (16)                            | (16)                   |
| <b>Iron</b> , mg                             | 0.05                              | 0.05                 | 0.05                            | 0.05                   |
| <b>Magnesium</b> , mg                        | 12                                | 11                   | 11                              | 12                     |
| <b>Manganese</b> , mg                        | Trace                             | Trace                | Trace                           | Trace                  |
| <b>Phosphorus</b> , mg                       | 103                               | 95                   | 98                              | 98                     |
| <b>Potassium</b> , mg                        | 144                               | 144                  | 155                             | 155                    |
| <b>Selenium</b> , µg                         | (1)                               | 1                    | (1)                             | (1)                    |
| <b>Zinc</b> , mg                             | 0.4                               | 0.4                  | 0.4                             | 0.4                    |

Source: McCance and Widdowson's The Composition of Foods. Fifth Edition. Ministry of Agriculture, Fisheries and Food, and The Royal Society of Chemistry (1991).

\* Will reduce if exposed to sunlight.

† Will be subject to degree of processing.

( ) Estimated value.

## 2.1 Milk and dairy products

### 2.1.1 Fluid milk products

#### 2.1.1.1 Beverage milks

Milk for direct human consumption must be passed heat treatment. Being a high nutritional value and complete food, it is an excellent medium for microbial growth. The heat treatment is used for destruction of microorganisms and increase storage life of milk. The fluid milk is heated after clarification to improve its durability and to kill pathogenic microorganisms. Heat treatments used can be classified as pasteurization, ultra high temperature, and sterilization.

##### 2.1.1.1.1 Pasteurization

Pasteurization is the most common practice of heat-processing milk for safety. The International Dairy Federation has defined pasteurization as “a process applied to a product with the object of minimizing possible health hazards arising from pathogenic microorganisms associated with milk, by heat treatment, which is consistent with minimal chemical, physical and organoleptic changes in the product” (Varnam and Sutherland, 1994). There are two main systems of milk pasteurization:

- **Low temperature long time (LTLT)** or batch process or low temperature holding method. The batch heating process involves bringing the milk to a suitable temperature, usually at 62-65 °C and holding it at that point for at least 30 minutes followed by rapid cooling to a temperature lower than 10 °C (Belitz and Grosch, 1987; Varnam and Sutherland, 1994).

- **High temperature short time (HTST)** or flash pasteurization. HTSH is the process of heating milk rapidly to a temperature of not less than 71 °C for 15-40 seconds (Belitz and Grosch, 1987; Varnam and Sutherland, 1994).

##### 2.1.1.1.2 Ultra high temperature (UHT) treatment

UHT milk was developed to meet the demand for milk to be stable for extended periods at room temperature and yet free of the unpleasant taste associated with sterilized milk. Plant for UHT milk may be placed in two categories:

- **Direct heating system.** The production of UHT milk by direct heating involves the mixing of superheated steam with milk, which is consequently heated almost instantaneously to the required sterilizing temperature at 140-150 °C for 2-4

seconds followed by aseptic packaging (Belitz and Grosch, 1987; Varnam and Sutherland, 1994; Vidal et al, 1993).

- **Indirect heating system.** A novel indirect method is carried out using tubular or plate heat exchangers similar to those used for pasteurization. The conditions used are 135-140 °C for 6-10 seconds (Belitz and Grosch, 1987; Varnam and Sutherland, 1994; McCarthy et al, 1985).

#### **2.1.1.1.3 Sterilization**

Sterilized milk is milk which is filtered or clarified and homogenized before maintaining at a temperature above 100 °C for a sufficient time to destroy all organisms present and is packaged in hermetically sealed containers. (Belitz and Grosch, 1987; Varnam and Sutherland, 1994).

#### **2.1.1.2 Cream**

During the separation of whole milk, two streams are produced: the fat-depleted stream, which produces the beverage milks as described above or skim milk for evaporation and possibly for subsequent drying, and the fat-rich stream, the cream. This usually comes off the separator with fat contents in the range of 35-45%. Cream is used for further processing in the dairy industry for the production of ice cream or butter, or can be sold to other food processing industries.

### **2.1.2 Concentrated and Dried Dairy Products**

Fluid milk contains approximately 88% of water. Concentrated milk products are obtained through partial water removal. Dried dairy products have even greater amounts of water removed to usually less than 4%. The benefits of both processes include an increased shelf-life, convenience, product flexibility, decreased transportation costs, and storage.

#### **2.1.2.1 Condensed milk**

The process is known as “condensing” or “evaporating” depending upon whether a vacuum pan or an evaporator is used as a means of removing the water. Condensed milk products include evaporated skim or whole milk, sweetened condensed milk, condensed buttermilk and condensed whey.

### **2.1.2.2 Dry milk or milk powder**

Drying denotes that the water in a liquid product is removed, so that the product acquires a solid form. The water content of milk powder ranges between 2.5 and 5% to ensure that no bacteria growth takes place at such low water content. Commercial methods of drying are based on heat being supplied to the product. The water is then evaporated and removed as steam. The residue is a dried product or milk powders. Two principal methods used for drying in the dairy industry involve roller drying and spray drying. Drying extends the shelf life of the milk, simultaneously reducing the weight and volume. This reduces the cost of product transportation and storage.

### **2.1.3 Cultured Dairy Products**

#### **2.1.3.1 Cheese**

Cheese is one type of cultured milk product. In this case, the starting material, milk is mixed with rennet and bacteria or other microorganisms to change lactose to lactic acid. Rennet is a mixture of enzymes that include rennin and pepsin. These enzymes make the milk curdle (clot or coagulate), leaving curds (the solid part) and whey (the liquid part). The curds are cut up and then lightly heated, causing them to shrink. The resulting cheese is allowed to age from 1 month (for example, brie) to 1 year (like cheddar). Some types of cheese can be consumed immediately, for example mozzarella.

#### **2.1.3.2 Yoghurt**

Yoghurt is a semi-solid fermented milk product which originated centuries ago in Bulgaria. Its popularity has grown and is now consumed in most parts of the world. The consistency, flavor and aroma may vary from one region to another. In some area yoghurt is produced in the form of a highly viscous liquid, whereas in other countries it is in the form of a softer gel. Yoghurt is usually classified as follow:

- Set type yoghurt, which is filled immediately after inoculation with bulk starter and incubated in the package
- Stirred type yoghurt, which is inoculated and incubated in the tank. After incubation the product is cooled before filling.

- Drink type yoghurt, which is based on the stirred type. The coagulum is broken down to liquid before filling.

#### **2.1.4 Ice cream**

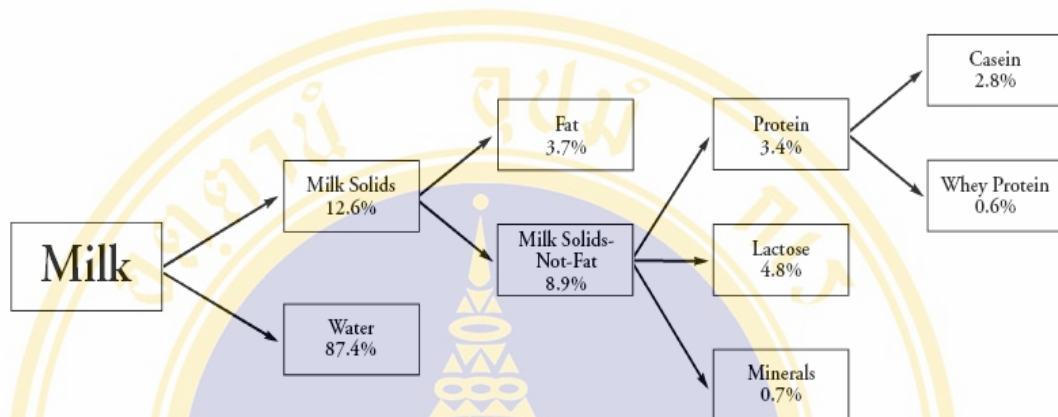
Ice cream and other frozen treats can be made from a variety of milks, buttermilk, condensed milk, cream or whey (the liquid part in cheese production). Ice cream must contain at least 10% fat and 20% milk solids to be called ice cream in the United States. Ice cream is a mixture of milk, sugar, cream, flavoring and stabilizer. The stabilizer is used to prevent ice crystals formation in the ice cream. The ingredients are mixed, pasteurized, and then homogenized to keep a smooth texture. The mix is then quickly cooled down to 40 °F.

#### **2.1.5 Butter**

Butter is a very concentrated form of milk. Butter is composed of 82% fat, 16% water and 2% milk solids. Fat in milk is suspended in the mixture by churning or agitating unhomogenized milk. Fat breaks out of the mixture in the size of rice grains. The butterfat clumps together and separates from the water, which is known as buttermilk.

## 2.2 Milk composition

The major constituents of milk are illustrated in Figure 1. Milk is made up of 87.4% water and 12.6% milk solids (3.7% fat, 8.9% milk solids-not-fat). The milk solids-not-fat contains protein (3.4%), lactose (4.8%), and minerals (0.7%).



Source: <http://www.nationaldairycouncil.org/Nutrition/Products/figure02.pdf>

Figure1 Major constituents of milk (Chandan R., 1997).

### 2.2.1 Water

Water content of milk is dependent upon the synthesis of lactose. Without some water in milk, it would be a viscous secretion composed mostly of lipid and protein and would be extremely difficult to remove from the gland. Water content of milk can be range from a low content in marine mammals to a high content in human milk and others. Cow milk contains approximately 87% water, so the transport of milk from the dairy farm to the processing plant involves hauling considerable amounts of water.

### 2.2.2 Lipids

Milk fat is a natural fat with unique physical, chemical, and biological properties. It contributes to the appearance, texture, flavor, and satiability of dairy foods. Milk fat is a source of energy, essential fatty acids, fat-soluble vitamins, and several other potential health-promoting components. Milk lipids are mainly triacylglycerols (triglycerides) or esters of fatty acids with glycerol (97-98%), 0.2 to 1.0% phospholipids, 0.2 to 0.4% free sterols (cholesterol, waxes, and squalene, an

intermediate of cholesterol), traces of free fatty acids, and varying amounts of the fat-soluble vitamins A, D, E, and K (Table 4).

Table 4 Composition of milk lipids

| Class of Lipid                     | Per 100 Grams Whole Fluid Milk (3.3% Fat) | Per Cup (8 oz., 244g) Whole Fluid Milk |
|------------------------------------|---|--|
| Vitamin A activity                 | 126 IU (31 RE)                            | 307 IU (76 RE)                         |
| Vitamin D                          | 1-3 IU                                    | 2.44 -7.32 IU                          |
| Vitamin E                          | 0.100 mg                                  | 0.244 mg                               |
| Vitamin K                          | 0.00334 mg                                | 0.008 mg                               |
| Triacylglycerols (Triglycerides)   | 3.23-3.27 g                               | 7.88-7.98 g                            |
| Diglycerides                       | 0.01-0.02 g                               | 0.02-0.05 g                            |
| Monoglycerides                     | 0.53-1.27 mg                              | 1.29-3.10 mg                           |
| Keto acid glycerides (total)       | 28.4-42.75 mg                             | 69.3-104.3 mg                          |
| Ketogenic glycerides               | 1.00-4.3 mg                               | 2.44-10.5 mg                           |
| Hydroxy acid glycerides (total)    | 20.0-26.05 mg                             | 48.8-63.56 mg                          |
| Lactogenic glycerides              | 2.00 mg                                   | 4.88 mg                                |
| Neutral glyceryl ethers            | 0.53-0.67 mg                              | 1.293-1.635 mg                         |
| Neutral plasmalogens               | 1.34 mg                                   | 3.270 mg                               |
| Free fatty acids                   | 3.34-14.7 mg                              | 8.15-35.87 mg                          |
| Phospholipids (total)              | 6.7-33.4 mg                               | 16.30-81.50 mg                         |
| Sphingolipids (less sphingomyelin) | 2.00 mg                                   | 4.88 mg                                |
| Free sterols                       | 7.35-13.69 mg                             | 17.93-33.40 mg                         |
| Cholesterol                        | 14 mg                                     | 33 mg                                  |
| Squalene                           | 0.2338 mg                                 | 0.570 mg                               |
| Carotenoids                        | 0.0233-0.0301 mg                          | 0.057-0.073 mg                         |

Source: Wong, et al., 1988 and USDA Nutrient Database.

More than 400 different fatty acids and fatty acid derivatives are found in milk, ranging from butyric acid with 4 carbon atoms to fatty acids with 26 carbon atoms. Milk fat is characterized not only by numerous different fatty acids, but also by their chain lengths. Among animal fats, milk fat is unique because it contains a relatively high proportion of short-chain and medium-chain saturated fatty acids, many of which are not found in other fats. Milk fat contains about 7% short-chain fatty acids (C4 to C8), 15 to 20% medium-chain fatty acids (C10 to C14), and 73-78% long-chain fatty acids (C16 and higher). Although the composition of milk fat varies

according to such factors as the breed of the cow and composition of the feed, the fatty acids in milk fat are approximately 65% saturated, 32% monounsaturated, 3% polyunsaturated, with minor amounts of other types of fatty acids (Table 5).

Table 5 Fatty acid composition of bovine milk lipid

| Type of Fatty Acid     | Percent of Total Fat | Fatty Acid             | Composition | Percent of Total Fat |
|------------------------|----------------------|------------------------|-------------|----------------------|
| <b>Saturated</b>       | 62.3                 | Butyric                | 4:0         | 3.3                  |
|                        |                      | Caproic                | 6:0         | 1.7                  |
|                        |                      | Caprylic               | 8:0         | 1.2                  |
|                        |                      | Capric                 | 10:0        | 2.3                  |
|                        |                      | Lauric                 | 12:0        | 2.6                  |
|                        |                      | Myristic               | 14:0        | 10.2                 |
|                        |                      | Palmitic               | 16:0        | 26.3                 |
|                        |                      | Stearic                | 18:0        | 12.0                 |
|                        |                      | <b>Monounsaturated</b> | 28.7        | Oleic                |
| <b>Polyunsaturated</b> | 3.6                  | Linoleic               | 18:2        | 2.4                  |

Source: [www.anslab.iastate.edu/Class/AnS270/milkfat.pdf](http://www.anslab.iastate.edu/Class/AnS270/milkfat.pdf)

### 2.2.3 Protein

Bovine's milk is a heterogeneous mixture of proteins (Table 6). About 80% of the total protein in milk is casein and 20% is whey protein. Milk also contains small amounts of various enzymes (e.g., lipoprotein lipase, alkaline phosphatase, lactoperoxidase) and traces of non-protein nitrogenous compounds (e.g., ammonia, urea, creatinine, creatine, and uric acid).

Casein, the dominant protein in bovine's milk, can be fractionated electrophoretically into four major components: alpha-, beta-, gamma-, and kappa-casein. Casein is generally defined as the protein precipitated at pH 4.6, a property used in the manufacturing of cheese. Whey protein, which is more heterogeneous than casein, consists predominantly of beta-lactoglobulin and alpha-lactalbumin. Alpha-lactalbumin has a high content of the amino acid tryptophan, a precursor of niacin. Other whey proteins present in smaller amounts are serum albumin, immunoglobulins (e.g., IgA, IgG, IgM), protease peptones, lactoferrin, and transferrin. Each of these proteins has unique characteristics.

Table 6 The concentration of proteins in milk.

|                     | grams/ liter | % of total protein |
|---------------------|--------------|--------------------|
| Total Protein       | 33           | 100                |
| Total Caseins       | 26           | 79.5               |
| alpha s1            | 10           | 30.6               |
| alpha s2            | 2.6          | 8.0                |
| beta                | 9.3          | 28.4               |
| kappa               | 3.3          | 10.1               |
| Total Whey Proteins | 6.3          | 19.3               |
| alpha lactalbumin   | 1.2          | 3.7                |
| beta lactoglobulin  | 3.2          | 9.8                |
| BSA                 | 0.4          | 1.2                |
| Immunoglobulins     | 0.7          | 2.1                |
| Proteose peptone    | 0.8          | 2.4                |

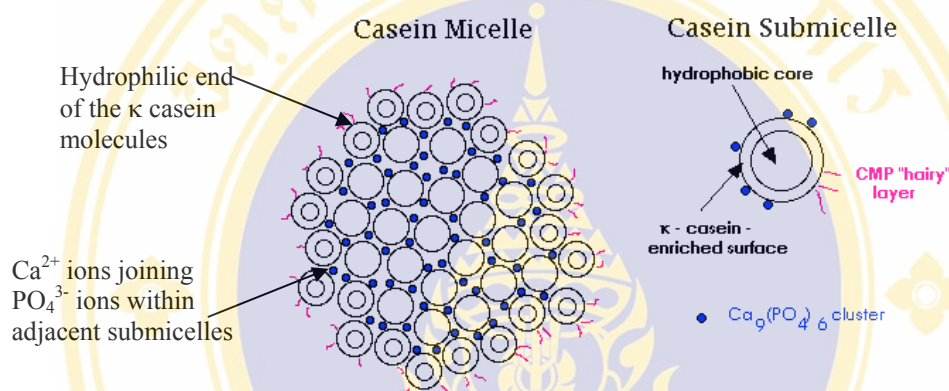
Source: Douglas, 1995

Casein is composed of several similar proteins which form a multi-molecular, granular structure called a casein micelle. In addition to casein molecules, the casein micelle contains water and salts (mainly calcium and phosphorous). Some enzymes are associated with casein micelles, too. Casein is one of the most abundant organic components of milk, along with the lactose and milk fat. Individual molecules of casein alone are not very soluble in the aqueous environment of milk. However, the casein micelle granules are maintained as a colloidal suspension in milk. If the micellar structure is disturbed, the micelles may come apart and the casein may come out of solution, forming the gelatinous material called the curd. This is part of the basis for formation of all non-fluid milk products. The main forms of casein present in milk are called  $\alpha$ ,  $\beta$  and  $\kappa$  (“kappa”) casein.

-  $\alpha$  and  $\beta$  casein are dominated by amino acids in which the side chains are insoluble in water but are attracted to each other (Figure 2). These “hydrophobic” forces cause the casein molecules to join in small particles called submicelles. The  $\kappa$  casein molecules also have a hydrophobic end which joins inside these submicelles.

- However one side of the  $\kappa$  casein molecule has a large number of amino acids containing the  $-OH$  group which attracts water and which protrude outside the submicelle as the 'hairy layer'. These "hydrophilic" forces enable the submicelle as a whole to dissolve in water.

- The submicelles contain phosphate  $PO_4^{3-}$  ions at the surface. Calcium  $Ca^{2+}$  ions can attach to the phosphate ions in adjacent submicelles, in effect 'gluing' the submicelles together into a much larger micelle.



Source: <http://class.fst.ohio-state.edu/FST822/lectures/Milk.htm>

Figure 2 Casein micelle and casein submicelle formed by  $\kappa$ -casein.

#### 2.2.4 Carbohydrate

The principal carbohydrate in milk is lactose, a natural disaccharide composed of the monosaccharides D-glucose and D-galactose, joined in a  $\beta$ -1, 4-glycosidic linkage. The chemical name for lactose is 4-O- $\beta$ -D-galactopyranosyl-D-glucopyranose. Lactose accounts for about 54% of the total solids-not-fat content of whole milk and about 30% of its calories (about 9% of the calories of 2% reduced fat milk). The lactose content of milk varies by species. Bovine's milk contains about 4.8% lactose (12 to 12.5% lactose/cup), whereas human milk has 7% lactose (15 to 18 g lactose/cup). Minor quantities of glucose, galactose, and oligosaccharides are also present in milk. Glucose and galactose are the products of lactose hydrolysis by the enzyme lactase. Carbohydrates other than lactose are found in milk, but at low concentrations. Low concentrations of free glucose (about 0.1 mM) and free galactose (about 0.2 mM) are found in cow milk and milk of other species. Other carbohydrates found free in milk include amino sugars, sugar phosphates, neutral and acid

oligosaccharides, and nucleotide sugars. Some of the complex oligosaccharides are thought to be important in helping establish the microflora of the neonate intestine (such as the bifidus factor identified in human milk).

### 2.2.5 Vitamins

Vitamins are organic substances essential for many life processes. Milk includes fat soluble vitamins A, D, E, and K (Table 7). Vitamin A is derived from retinol and  $\beta$ -carotene. Because milk is an important source of dietary vitamin A, fat reduced products which have lost vitamin A with the fat are required to supplement the product with vitamin A.

Milk is also an important source of dietary water soluble vitamins, i.e. B1–thiamine, B2–riboflavin, B6–pyridoxine, B12–cyanocobalamin, niacin, and pantothenic acid

There is also a small amount of vitamin C (ascorbic acid) presents in raw milk but is very heat-labile and easily destroyed by pasteurization.

Table 7 The vitamin content of fresh milk.

| Vitamin               | Contents per litter |
|-----------------------|---------------------|
| A (ug RE)             | 400                 |
| D (IU)                | 40                  |
| E (ug)                | 1000                |
| K (ug)                | 50                  |
| B1 (ug)               | 450                 |
| B2 (ug)               | 1750                |
| Niacin (ug)           | 900                 |
| B6 (ug)               | 500                 |
| Pantothenic acid (ug) | 3500                |
| Biotin (ug)           | 35                  |
| Folic acid (ug)       | 55                  |
| B12 (ug)              | 4.5                 |
| C (mg)                | 20                  |

Source: Douglas, 1995

### 2.2.6 Minerals

All 22 minerals considered to be essential to the human diet are present in milk (Table 8). These include three families of salts:

1. Sodium (Na), Potassium (K) and Chloride (Cl): These free ions are negatively correlated to lactose to maintain osmotic equilibrium of milk with blood.
2. Calcium (Ca), Magnesium (Mg), Inorganic Phosphorous (P(i)), and Citrate: This group consists of 2/3 of the Ca, 1/3 of the Mg, 1/2 of the P(i), and less than 1/10 of the citrate in colloidal (no diffusible) form and present in the casein micelle.
3. Diffusible salts of Ca, Mg, citrate, and phosphate: These salts are very pH dependent and contribute to the overall acid-base equilibrium of milk.

Table 8 The mineral content of fresh milk

| Mineral         | Content per liter |
|-----------------|-------------------|
| Sodium (mg)     | 350-900           |
| Potassium (mg)  | 1100-1700         |
| Chloride (mg)   | 900-1100          |
| Calcium (mg)    | 1100-1300         |
| Magnesium (mg)  | 90-140            |
| Phosphorus (mg) | 900-1000          |
| Iron (ug)       | 300-600           |
| Zinc (ug)       | 2000-6000         |
| Copper (ug)     | 100-600           |
| Manganese (ug)  | 20-50             |
| Iodine (ug)     | 260               |
| Fluoride (ug)   | 30-220            |
| Selenium (ug)   | 5-67              |
| Cobalt (ug)     | 0.5-1.3           |
| Chromium (ug)   | 8-13              |
| Molybdenum (ug) | 18-120            |
| Nickel (ug)     | 0-50              |
| Silicon (ug)    | 750-7000          |
| Vanadium (ug)   | tr-310            |
| Tin (ug)        | 40-500            |
| Arsenic (ug)    | 20-60             |

Source: Douglas, 1995

### 2.2.7 Milk enzymes

Milk is also rich in native enzymes, and about 50 different enzyme activities have been reported. However, only a small number of enzymes are of practical significant.

The principal lipase in milk is lipoprotein lipase, an enzyme which catalyses the hydrolysis of triglycerides to free fatty acid. This reaction results in the production of soapy, bitter, rancid and unclean flavors in dairy products. The enzyme is present in freshly drawn milk in great excess and, under certain conditions, spoils the milk within a few minutes. Spontaneous lipolysis is sometimes encountered in raw milk and seems to be influenced by a number of factors. These include the stage of lactation, season, diet and plane of nutrition.

The major proteinase in milk is plasmin, a serine proteinase with trypsin-like activity. At neutral pH values, milk plasmin is very heat stable. The enzyme survives pasteurization, and residual activity can still be traced after UHT sterilization.

Lactoperoxidase is another milk enzyme that is present at high concentrations. It catalyses the transfer of oxygen from hydrogen peroxide to other substrates such as thiocyanate. Lactoperoxidase has the potential to catalyse oxidation of unsaturated fatty acids, leading to the development of oxidized flavors. On the other hand, if the levels of thiocyanate and peroxide in milk are supplemented, lactoperoxidase acts as a powerful bacteriocide which can kill coliforms, Salmonellae, Shigellae and Pseudomonads.

Xanthine oxidase is present in milk and can catalyse non-specific oxidation of dairy products. Nevertheless, its overall significance is unlikely to be high.

Although not a degradative enzyme, alkaline phosphatase is worthy of mention since it is almost completely inactivated by pasteurization, and is therefore used as an index of the efficiency of such heat treatments.

## 2.3 Physical properties of milk

The physical properties of milk are summarized in Table 9

### 2.3.1 Density

Density, the mass of a certain quantity of material divided by its volume, is dependant on the following factors:

- Temperature at the time of measurement
- Temperature history of the material
- Composition of the material (especially the fat content)
- Inclusion of air (a complication with more viscous products)

The density of milk normally varies between 1.028 and 1.034 kg/l, depending on the composition. Milk is therefore only slightly denser than water (Alfa-Laval, 1995)

### 2.3.2 Viscosity

Viscosity of milk and milk products is important in determining the following characteristics:

- The rate of creaming
- Rates of mass and heat transfer
- The flow conditions in dairy processes

Milk and skim milk, excepting cooled raw milk, exhibit Newtonian behavior in which the viscosity is independent of the rate of shear. The viscosity of these products depends on the following factors:

Temperature:

- Cooler temperatures increase viscosity due to the increased voluminosity of casein micelles
- Temperatures above 65° C increase viscosity due to the denaturation of whey proteins

pH:

- An increase or decrease in pH of milk also causes an increase in casein micelle voluminosity.

Cooled raw milk and cream exhibit non-Newtonian behavior in which the viscosity is dependant on the shear rate. Agitation may cause partial coalescence of the fat globules (partial churning) which increases viscosity. Fat globules that are under gone cold agglutination, may be dispersed due to agitation, causing a decrease in viscosity.

### **2.3.3 Freezing point**

Freezing point is a colligative property, which is determined by the molarity of solutes rather than by the percentage by weight or volume. In the dairy industry, freezing point is mainly used to determine added water but it can also been used to determine lactose content in milk, to estimate whey powder contents in skim milk powder, and to determine water activity of cheese. The freezing point of milk is usually in the range of  $-0.512$  to  $-0.550$  °C with an average of about  $-0.522$  °C (Douglas, 1995).

### **2.3.4 Acid-Base Equilibrium**

Both titratable acidity and pH are used to measure milk acidity. The pH of milk at 25° C normally varies within a relatively narrow range of 6.5 to 6.7. The normal range for titratable acidity of milks is 13 to 20 mmol/l. Because of the large inherent variation, the measure of titratable acidity has little practical value except to measure changes in acidity (eg, during lactic fermentation) and even for this purpose, pH is a better measurement.

Table 9 General physical properties of milk

| Property                   | Value                | Definition and Significance   | Property                    | Value                      | Definition and Significance   |
|----------------------------|----------------------|---|-----------------------------|----------------------------|---|
| Titrateable acidity, % max | 0.16                 | The total acidity or the amount of alkali required to neutralize the acidic constituents. Generally expressed as lactic acid. Used to determine bacterial growth in fermentations and compliance standards.   | Specific heat at            |                            | The specific heat of milk products depends on their composition and the temperature. Important in processing as the amount of heat or refrigeration required may be calculated from the weight and specific heat of the different products being pasteurized or cooled.   |
|                            |                      |   | 0° C                        | 0.92                       |   |
|                            |                      |   | 15° C                       | 0.94                       |   |
|                            |                      |   | 40° C                       | 0.93                       |   |
| pH                         | 6.6 ± 0.2 at 25° C   | Fresh milk is slightly acid (pH of drinking water is 7.0-8.5). Generally the pH is lower (pH 6.0) in colostrum and higher (up to 7.5) during mastitis than in normal milk of mid-lactation.   | Coefficient of expansion at |                            | The ratio of an increase in volume per unit increase in temperature. Milk expands when heated and contracts when cooled. Used for design of dairy equipment.  |
|                            |                      |   | 10° C                       | 0.9975                     |   |
|                            |                      |   | 15.6° C                     | 0.9985                     |   |
|                            |                      |   | 21.1° C                     | 1.0000                     |   |
| Surface tension            | 50-52 dynes at 20° C | Normally, cow's milk's surface tension is about 70% of that of water. Involved in adsorption and formation and stability of emulsions. Important to creaming, functions of fat globule membranes, foaming, and emulsifier use.  | Viscosity                   | 2.0-2.1 cp at 20° C        | Refers to resistance to flow measured in centipoise (cp). Used to assess aggregation of protein micelles or fat globules. Also used for design of dairy equipment.  |
| Specific gravity           | 1.032 at 15° C       | Ratio of the density of the product and the density of water at the same temperature. Many milk constituents have a specific gravity (sg) greater than that of water which has a sg of one. The more fat in milk, the lower the sg as fat has an sg less than one. Used to estimate solids not fat. | Electrical conductivity     | 45-55x10 <sup>-4</sup> mho | In milk, fat and colloiddally dispersed substances decrease conductivity. Used to detect added neutralizers, follow fermentation, and monitor demineralization of whey.   |
| Freezing point             | -0.540° C            | Lower than that of pure water (0° C) due to dissolved substances in milk. Used to detect adulteration of milk with water.   | Osmolality*                 | 275 m Osm/kg               | The osmolality of a solution is based on the number of particles in solution – the greater the number of particles, the higher the osmolality. Osmolality of foods is important in planning diets of low osmolality for certain patients. Since a solution of lower osmolality requires transfer of less water to the stomach and gastrointestinal tract to dilute it, it should be better tolerated than one of higher osmolality. |
| Boiling point              | 100.17° C            | Greater than that of pure water (100° C) due to dissolved substances in milk. Used to detect adulteration of milk with added water.   |                             |                            |   |

\* Source: The Doyle Pharmaceutical Company, Minneapolis, Minn.

## 2.4 Effects of heat treatment on milks and their properties

The effects of heat treatment on the components of milk (proteins, lipids, carbohydrates and minerals) are very important for the final product character, since they undergo modifications that affect the sensory and nutritional quality of milk (Burton, 1984). The effects of the wide range of technological processes used in the dairy industry may be evaluated by determining several chemical compounds specifically related to such processes, either through degradation of original milk components or as a result of reactions at the high temperatures used (Francisco et al, 2000). There are two groups of reactions that may be used to assess heat treatment.

- Type I includes the denaturation, degradation and inactivation of heat-labile components, these are mainly whey proteins, enzymes and vitamins.

- Type II reactions includes the formation of new substances which are not present, or only at trace levels, in the unprocessed milk including lactulose or products of the maillard reaction e.g. furosine, hydroxymethylfurfural (Pellegrino et al, 1995; Mortier et al, 2000).

### 2.4.1 Heat-induced changes in milk related to coagulation

The coagulation of milk on extended heating at high temperatures (120–140 °C) is a consequence of loss of casein micelle stability, as a result of numerous physical and chemical changes in its components. The surface of the micelle has a number of dissociated carboxyl and some ester phosphate groups, providing a high negative charge and thus electrostatic stabilization. Then there is a diffuse surface layer of flexible, hydrophilic polypeptide chains consisting mostly of C-terminal segments of  $\kappa$ -casein, providing steric stabilization (Holt 1992).

Several factors influence the colloidal stability of milk. Important factors are calcium ions and pH, both of which diminish electrostatic repulsions and possibly alter the conformation of  $\kappa$ -casein at the micelle surface (indirectly reducing steric repulsions). Heat treatment markedly changes the serum phase environment around the casein micelles (e.g. change in pH and soluble minerals, in particular calcium ions, breakdown of lactose and urea) as well as the casein micelles themselves (association of whey proteins, dephosphorylation, casein dissociation). Some of these changes are listed in Table 10 (Singh, 2004).

Table 10 Changes in milk during heating and their possible impact on heat stability

| Changes that promote instability  | Changes that enhance stability  |
|---|---|
| <ul style="list-style-type: none"> <li>- Decrease in pH</li> <li>- Deposition of calcium phosphate onto micelles</li> <li>- Association of whey protein with casein micelles</li> <li>- Dephosphorylation of casein</li> <li>- Dissociation and hydrolysis of casein, in particular <math>\kappa</math>-casein</li> <li>- Reduction in zeta potential and hydration</li> <li>- Covalent bond formation</li> </ul> | <ul style="list-style-type: none"> <li>- Reduction in calcium ion activity</li> <li>- Association of whey proteins casein micelles</li> <li>- Reduced sensitivity of casein to calcium ions</li> <li>- Thermal degradation products of lactose</li> </ul> |

#### 2.4.2 Changes in casein micelles

Heating milk at the heat stability assay temperatures causes denaturation of whey proteins and their interactions with casein micelles.  $\kappa$ -Casein on the surface of casein micelles is involved in the formation of a specific disulphide-linked complex with  $\beta$ -lactoglobulin (Singh and Fox 1987a; Jang and Swaisgood 1990). Concentration of milk prior to heating has a marked effect on the dissociation of  $\kappa$ -casein. The extent of dissociation of  $\kappa$ -casein at any particular pH increases with the dissociation–pH curve shifting towards lower pH values (Nieuwenhuijse *et al.* 1991; Singh and Creamer 1991a). Most of the dissociated  $\kappa$ -casein was covalently linked to whey proteins (Singh, 2004). The pH of heating has a large effect on the extent of the association of whey proteins with the casein micelles (Smits and van Brouwershaven 1980; Singh and Fox 1985a, b). At pH values < 6.8, a majority of whey protein complexes remain associated with the casein micelle surface whereas at higher pH values, these complexes remain in the serum. On heating at pH values > 6.8, not only do the whey protein aggregates remain in the serum but also micellar  $\kappa$ -casein dissociates in the serum (Singh, 2004).

### 2.4.3 Changes in the serum phase

A crucial change in the serum is the decrease in pH, which plays a key role in creating an environment that favors coagulation of the milk proteins (Fox, 1981b). Heat treatment has been shown to reduce the concentration of soluble phosphate and of both soluble and ionic calcium. The calcium ion activity, which depends on the initial pH of the milk, also decreases upon heating. This decrease is reversible and some or all of the calcium ion activity is recovered after heating. (Van Boekel et al. 1989a,b). Another factor of importance in the serum phase is the whey proteins. These proteins are easily denatured by heat treatments above 70 °C and then react with each other or casein micelles (Singh, 2004). The major mechanism is the formation of intermolecular disulfide bonds by sulfhydryl–disulfide interchange (Havea et al, 2001), especially by  $\beta$ -lactoglobulin ( $\beta$ -Lg), which dominates the aggregation behavior of whey protein (Havea et al, 2001; Schokker et al, 2000a, b) by forming oligomers that combine into large aggregates (Fuente et al, 2002; Havea et al, 2001; Schokker et al, 2000a; Bon et al, 2002; Hong and Creamer, 2002).

### 2.4.4 Effects on vitamins

The fat soluble vitamins A, D, and E and the vitamin B2, pantothenic acid, biotin, and nicotinic acid are relatively insensitive to heat. There are generally no losses of these vitamins when milk is heated. However, vitamins B1, B6, B12, folic acid, and vitamin C, on the other hand, are less stable to heat as shown in Table 11 (Kessler and Horak, 1981; Zadow, 1980).

Table 11 Effect of difference methods of heat treatment on the vitamin losses

| Procedure      | Losses (%) of |            |             |            |           |
|----------------|---------------|------------|-------------|------------|-----------|
|                | Vitamin B1    | Vitamin B6 | Vitamin B12 | Folic acid | Vitamin C |
| Pasteurization | <10           | 0-8        | <10         | <10        | 10-25     |
| UHT treatment  | 0.20          | <10        | 5-20        | 5-20       | 5-30      |
| Boiling        | 10-20         | 10         | 20          | 15         | 15-30     |
| Sterilization  | 20-50         | 20-50      | 20-100      | 30-50      | 30-100    |

Source: Renner, 1983

### 2.4.5 Effects of heat via Maillard reaction

Heat treatment results in many chemical and structural changes in milk, the extent of which depends on the temperature and duration of heating (Evangelisti et al, 1999; Villamiel et al, 1996). One mechanism involved in the deleterious consequence of heat treatment is the Maillard reaction that leads to the decreased in nutritional value of proteins and formation of brown compounds in milk. The Maillard reaction can be divided into three stages: the early Maillard reaction, the advanced Maillard reaction and the final Maillard reaction (Mauron, 1981).

The early Maillard reaction consists of condensation of the reducing sugar with the amino group and leads, via formation of a Schiff's base and the Amadori rearrangement, to the so-called Amadori product (Figure 3). In milk, this Amadori product is lactulosyllysine (bound to protein).

The advanced Maillard reaction consists of the breakdown of the Amadori product (or other products related to the Schiff's base) into numerous fission products of the sugar-amino compound (Figure 4 and 5).

The final Maillard reaction consists of the condensation of amino compounds and sugar fragments into polymerized protein and brown pigments, called the melanoidins.

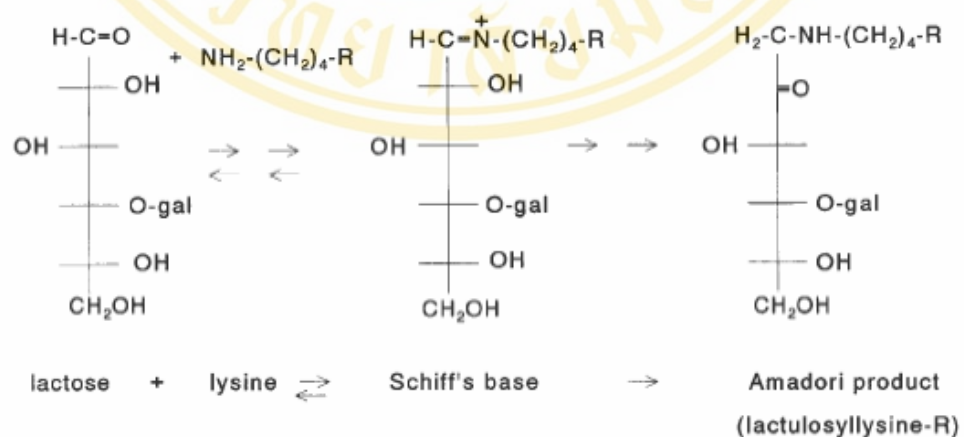


Figure 3 Schematic overview of the early Maillard reaction in milk, leading to Amadori product (gal=galactose, R=protein chain)

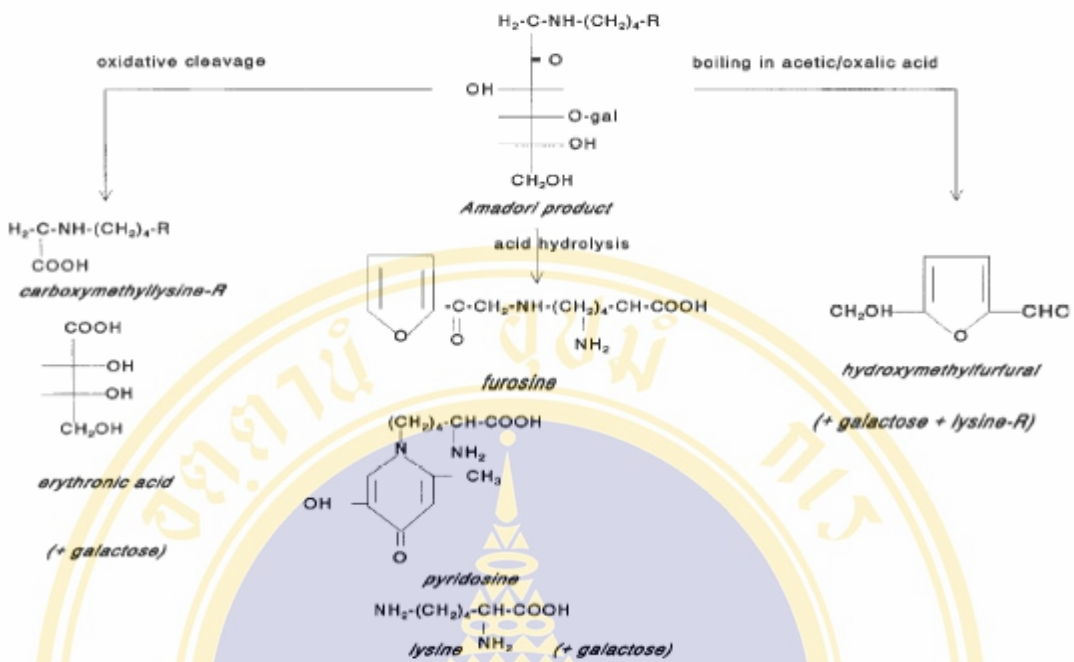


Figure 4 Degradation of the Amadori product via oxidative cleavage, acid hydrolysis, or boiling in acetic/oxalic acid (gal=galactose, R=protein chain).

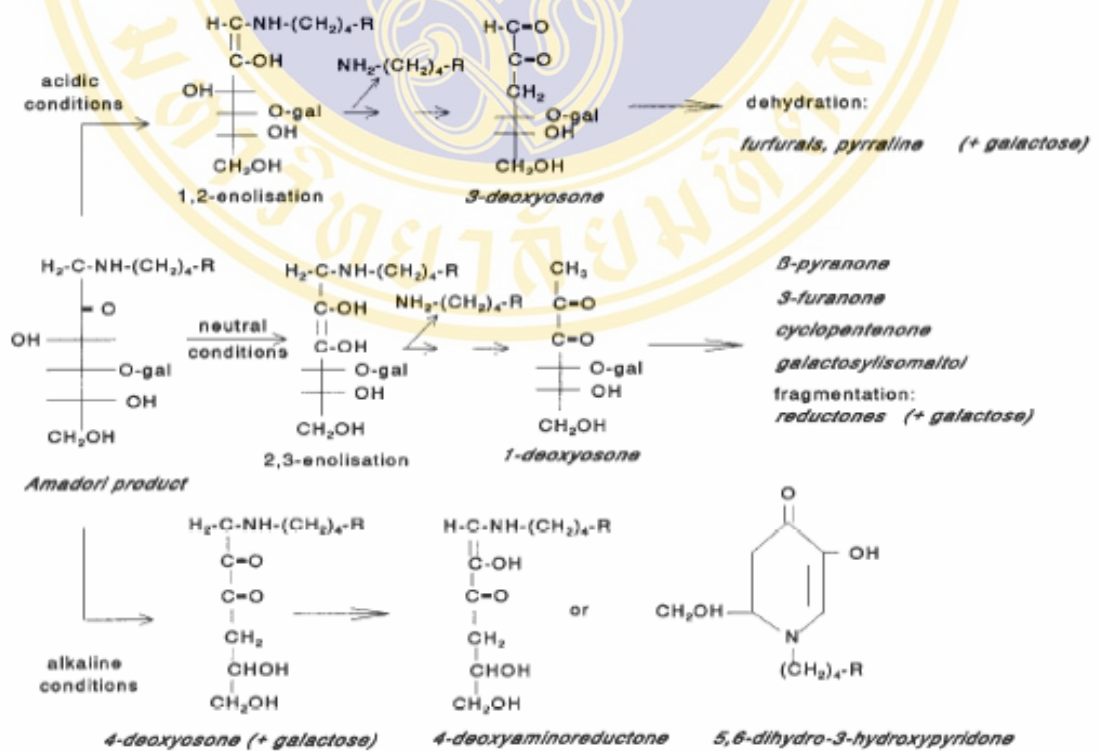


Figure 5 Breakdown of the Amadori product in the advanced Maillard reaction under acidic, neutral and basic conditions (gal=galactose, R=protein chain).

The consequences of the Maillard reaction for milk and milk products are considerable:

(a) Loss of nutritive value due to blockage of lysine residues, which are no longer available for digestion (early, advanced and final Maillard reaction, Finot, 1990), reduced digestibility and inhibition of enzymes (Friedman, 1996a,b)

(b) Flavor compounds are formed. These are mainly low molecular fission compounds from degradation of the Amadori compound (advanced Maillard reaction, e.g. Danehy, 1986)

(c) Antioxidative compounds are formed in the advanced Maillard reaction (Namiki, 1988; Bressa et al., 1996)

(d) Mutagenic (Shibamoto, 1982), as well as anti-mutagenic (Kato et al., 1987; Yen et al., 1992) and anticarcinogenic compounds (Aeschbacher, 1990), are formed. However, it should be noted that Berg *et al.* (1990) could not detect any mutagenicity in normally heat-treated milk, only in milk burnt to the pan. Formation of hetero- cyclic amines during heating (Jagerstad et al., 1991) is not relevant for milk, on the one hand because heating temperatures applied in dairy technology are too low for formation of hetero- cyclic amines, and on the other hand because an important precursor for heterocyclic amines, creatin(in)e is not present in significant quantities in milk.

(e) Antibacterial compounds may be formed (Einarsson et al., 1983).

(f) Antigenicity of heated cow's milk may be less for people allergic to cow's milk (Friedman, 1996a).

(g) Milk proteins polymerize because of the Maillard reaction (e.g. Zin El-Din et al., 1991; Ames, 1992; Zin El-Din and Aoki, 1993; Henle et al., 1996).

(h) A brown color develops due to melanoidins (advanced and final Maillard reaction, e.g. Patton, 1955; Namiki, 1988; Ames, 1992).

The extent of the reaction can be evaluated by the determination of the content of furosine ( $\epsilon$ -N-2-furoylmethyl-L-lysine), the main stable Amadori compound in the early Maillard reaction, and lactulose (4-O- $\beta$ -galactopyranosyl-D-fructose), respectively. Furosine and lactulose can be used as indicators of heat damage of milk and for distinction of UHT milk, pasteurized milk and in-container sterilized milk

(Acquistucci et al, 1996; Andrew, 1984; Corzo et al, 1994a; Corzo et al, 1994b; De Rafael et al, 1997; Geier and Klostermeyer, 1983; Villamiel and Corzo, 1998). The combination of lactulose and furosine values for UHT milk provides information on the quality and genuineness of commercial milks (Pellegrino et al, 1995), particularly to detect the presence of reconstituted milk powder in consumption milk (Ding and Chang, 1986; van Renterghem and De Block, 1996).



## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Chemicals

- Gracial acetic acid was purchased from Lab-Scan, Ireland.
- Butanol was purchased from APS, Australia.
- Sodium hydroxide and urea was from Merck, Germany.
- 2, 3, 5-Triphenyltetrazolium chloride was purchased from Fluka, Switzerland. All chemicals were analytical grade.

#### 3.2 Equipment

- Filter paper was purchased from Whatman, England.
- Hot plate stirrer was from Fisher Scientific, USA.
- Mastersizer 2000 was from Malvern, UK.
- Milkoscan™ 4000 was from Foss Electric, Denmark.
- Pipetman 1 ml and 5 ml. were from Gilson Medical Electronics, France.
- Shaking water bath was from Heto SBD-50, Denmark.
- Spectronic was from Spectronic Unicam, USA.

#### 3.3 Milk samples

Three types of milk samples were used in this experiment i.e. raw milk, milk powder, and market fresh milk.

3.3.1 Raw milk samples were obtained from Zonta Chombung Cooperative in Ratchaburi Province. They were used within 24 hour after milk collection.

3.3.2 Milk powder was purchased from a supermarket in Bangkok. There were two types of milk sample used i.e. whole milk powder, and skimmed milk powder

3.3.3 Market fresh milk was purchased from a supermarket in Bangkok. There were two types of market fresh milk used in this study i.e. pasteurized milk, and UHT milk.

### 3.4 Preparation of milk samples

Whole milk powder and skimmed milk powder was reconstituted with distilled water. For reconstituted whole milk, 167.0 g. of whole milk powder was dissolved in 1.0 L. of distilled water. Reconstituted skimmed milk was prepared by dissolving 150.0 g. of skimmed milk powder in 1.0 L. of distilled water. Raw milk and reconstituted milk (whole milk and skimmed milk) were mixed for varied percentage of milk powder as shown in Table 12.

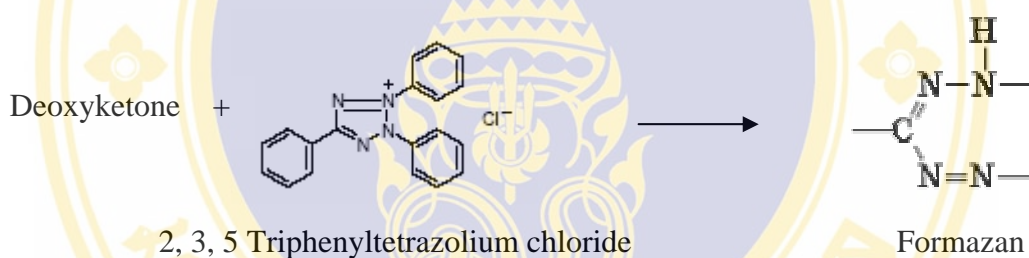
Table 12 Ratio of milk samples as varied percentage of reconstituted milk

| <b>% milk powder</b> | <b>Raw milk<br/>(ml.)</b> | <b>Reconstituted milk<br/>(ml.)</b> |
|----------------------|---------------------------|-------------------------------------|
| 0                    | 100                       | 0                                   |
| 5                    | 95                        | 5                                   |
| 10                   | 90                        | 10                                  |
| 15                   | 85                        | 15                                  |
| 20                   | 80                        | 20                                  |
| 30                   | 70                        | 30                                  |
| 40                   | 60                        | 40                                  |
| 50                   | 50                        | 50                                  |
| 60                   | 40                        | 60                                  |
| 70                   | 30                        | 70                                  |
| 80                   | 20                        | 80                                  |
| 90                   | 10                        | 90                                  |
| 100                  | 0                         | 100                                 |

The combination of milk samples were analyzed for deoxyketone, biochemical analysis, ratios of milk composition, and milk fat particle size analysis. Pasteurized and UHT milk samples purchased from supermarkets were subjected to deoxyketone determination, biochemical analysis, ratios of milk composition, and milk fat particle size analyses.

### 3.5 Determination of deoxyketone in milk samples

The principle for deoxyketone determination is based on colorimetric reaction. Deoxyketone occur in maillard reaction can reduced 2, 3, 5 Triphenyltetrazolium chloride (TTC) to formazan, which can be determined by spectrophotometer.



The combination of milk samples and market fresh milk were analyzed for deoxyketone content by a total volume 10.0 ml. of milk sample was pipetted into 50 ml. Erlenmeyer flask and 1.0 ml. of 10% acetic acid was added, the resulting solution was stirred gently for 5 minutes. Place the Erlenmeyer flask into a water bath at 40.0°C for 10 minutes then filter through a paper filter. Rinse carefully the precipitate at least 200 ml. with 40.0°C distilled water and then weigh 0.5 g. of filtrate into test tube and 5.0 ml. of saturated urea solution was added. The solution was then well mixed and place into a water bath at 40.0°C for 5 minutes. Add 1.0 ml. of TTC (2, 3, 5-Triphenyltetrazolium chloride), alkaline solution shake and place into a water bath at 40.0°C for 10 minutes. Follow by 1.0 ml. of acetic acid and 5.0 ml. of 1-butanol were added. Mixed for 5-10seconds and measured the upper phase by spectrophotometer at 486 nm.

### 3.6 Biochemical analysis of milk samples

A total volume of 20.0ml of milk samples was poured to plastic vial and preheated to 40 °C for 15 minute to melt the fat and make it homogeneous before fat, protein, lactose, urea, Cit.Ac, SNF, and TS analysis by MilkoScan™ 4000 (Foss, Denmark).



Figure 6 MilkoScan™ 4000 for milk proximate analysis

The principle of MilkoScan™ 4000 is based on infrared analysis. MilkoScan™ 4000 works with the mid-Infrared region of the spectrum from 3 - 10  $\mu\text{m}$  (Figure 7) corresponding to 1000 – 5000  $\text{cm}^{-1}$ .

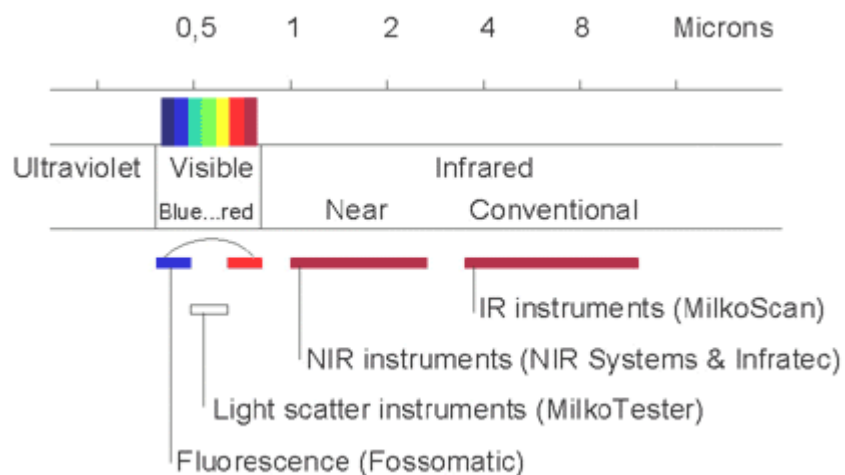


Figure 7 The electromagnetic spectrum and some region for milk analysis

The infrared analysis principle employed in the filter instruments is based on the well-proven Single Beam Infrared System. An infrared source provides light spanning the range of the infrared region. The light passes through different filters that only admit certain specific wavelengths, corresponding to absorption frequencies of the components in question. Then the light passes through a cuvette containing the product sample and finally hits the detector (Figure 8).

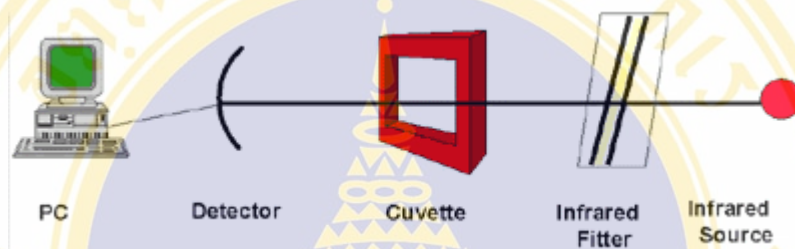


Figure 8 Schematic for the IR principle

### The four main infrared filters

#### Fat

The fat molecule consists of a glycerol backbone to which three fatty acids are bound. Two different fat filters are used in the IR instruments, Fat A and Fat B.

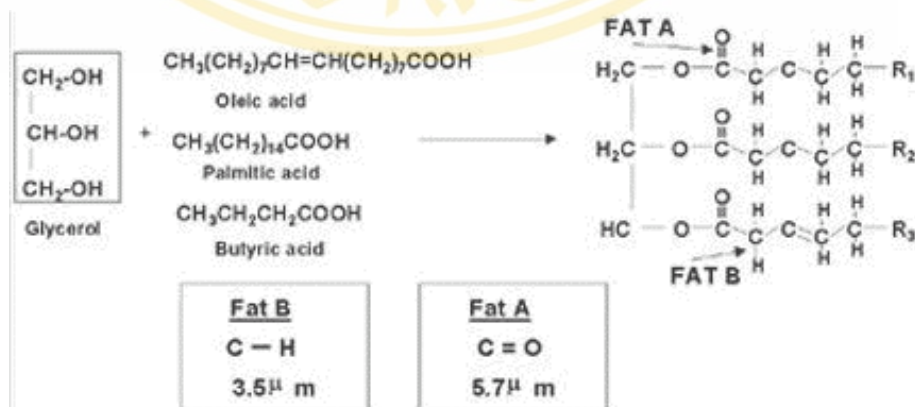


Figure 9 Fat A and Fat B molecule

**Fat A** absorption is due to stretching vibrations in the C=O bond of the carbonyl group in fat. This filter counts the number of fat molecules regardless of the length and the molecular weight of the individual fatty acids. This is a drawback if the length of the fatty acids changes.

**Fat B** absorption is due to stretching vibrations in the C-H bonds of fatty acid chains. The measurement is therefore related both to the size and to the number of fat molecules in the sample.

### Lactose

The lactose molecule is a disaccharide consisting of two monosaccharides glucose and galactose joined together. The hydroxyl group, (-OH) is a characteristic of carbohydrates and the lactose absorption is due to the C-OH bond.

### Protein

The protein molecule consists of amino acid units joined together with peptide bonds. Protein absorption is due to stretching vibrations in the N-H bonds within the peptide bonds.

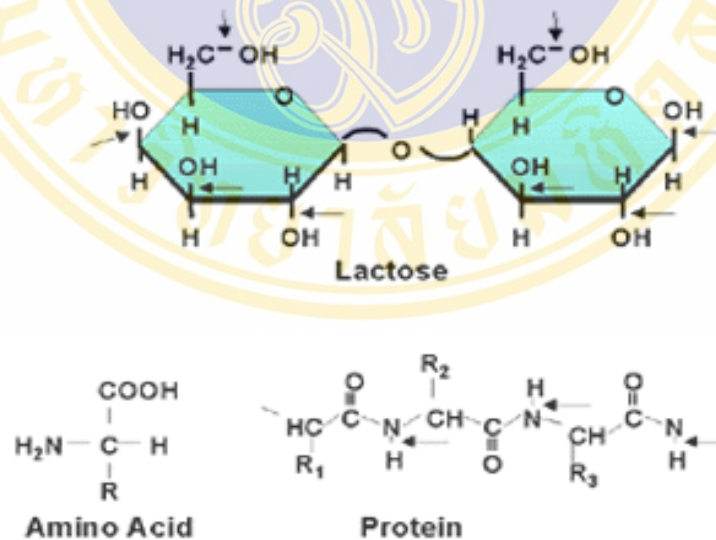


Figure 10 The protein and lactose molecules

In addition to the four main filters mentioned about, specific filters for urea, citric acid and H-index are also included in the instrument. Reference filters to stabilize readings of the main filters are present in MilkoScan™ 4000.

### 3.7 Determination of ratios of milk compositions in milk samples

From the results of biochemical analysis the ratios of milk compositions were calculated using the values of every component for determining suitable factors that could differentiate pure fresh milk and fresh milk combined with reconstituted milk powder (whole milk powder and skimmed milk powder).

### 3.8 Determination of milk fat particle size in milk samples

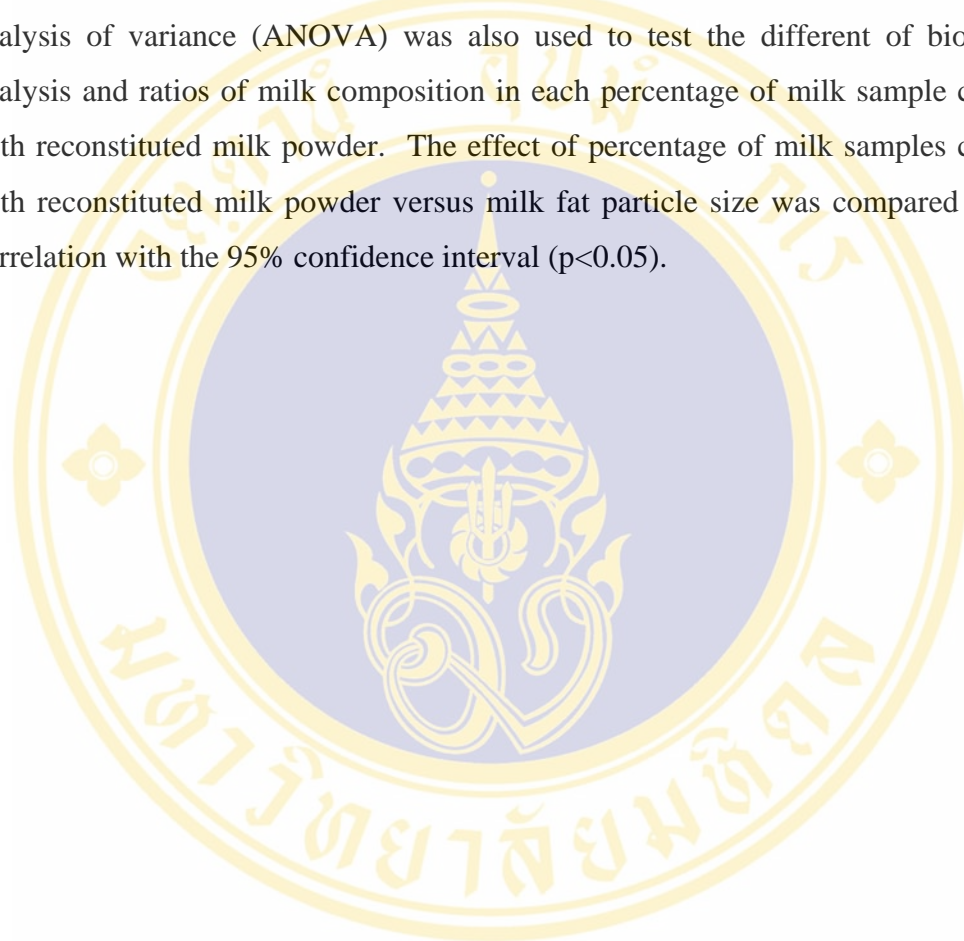
Milk samples were subjected to milk fat particle size measurement using Mastersizer 2000 based on the Mie theory. This theory employs knowledge of the light refractive index of the particles and the dispersion media and the imaginary part of the refractive index of the particles. The pattern of diffraction light (He-Ne laser) is dependent on the particle size. The laser diffraction pattern is measured and correlated to the particle size distribution based on Fraunhofer or Mie theory.



Figure 11 Mastersizer 2000 for milk fat particle size analysis.

### 3.9 Statistical analysis

The effect of percentage of milk powder versus the deoxyketone content of raw milk combined with reconstituted milk powder (whole milk powder and skimmed milk powder) and market fresh milk (pasteurized milk and UHT milk) were compared using analysis of variance (ANOVA) with the 95% confidence interval ( $p < 0.05$ ). The analysis of variance (ANOVA) was also used to test the different of biochemical analysis and ratios of milk composition in each percentage of milk sample combined with reconstituted milk powder. The effect of percentage of milk samples combined with reconstituted milk powder versus milk fat particle size was compared by using correlation with the 95% confidence interval ( $p < 0.05$ ).



## CHAPTER IV

### RESULTS

#### 4.1 Determination of deoxyketone in milk samples

The absorbances of deoxyketone determination in raw milk combined with reconstituted whole milk powder and skimmed milk powder are shown in Table 13 and Table 14. In this study, the absorbances of deoxyketone in raw milk combined with reconstituted whole milk powder were increased as the percentage of milk powder increased. The increment of the absorbance of deoxyketone in raw milk combined with reconstituted skimmed milk powder was in the same manner but with higher slope. The absorbance value of deoxyketone contents in pasteurized milk samples and UHT milk samples were presented in Table 15 and Table 16. In pasteurized milk samples, the average absorbance of deoxyketone content in pasteurized whole fresh milk (n=5) was 1.34 with ranging from 1.27 to 1.52. The results indicated that milk samples might be produced by combined raw milk and reconstituted whole milk powder or combined raw milk and reconstituted skimmed milk powder in a range of 30 to 50 percent or 15 to 30 percent, respectively. In pasteurized low-fat milk samples (n=5), the average absorbance of deoxyketone content was 1.34 ranging from 0.81 to 2.03. These indicated that pasteurized low-fat milk samples investigated might be produced by combining raw milk with 15 to 80 percent of reconstituted whole milk powder or might be combining raw milk with 5 to 40 percent of reconstituted skimmed milk powder.

In UHT whole fresh milk (n=7) and UHT low-fat milk (n=2) samples, the average absorbance of deoxyketone content was 2.807, ranging from 1.90 to 3.80. The results indicated that most of the UHT milk samples by investigated were produced by combining raw milk with reconstituted whole milk powder or skimmed milk powder at high levels (60-100% and 30-80%), respectively.

Table 13 The absorbance of deoxyketone content in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder.

| % WMP*       | Average absorbance<br>at 486 nm. | SD    |
|--------------|----------------------------------|-------|
| 100% RM      | 0.32                             | 0.034 |
| RM + 5% WMP  | 0.49                             | 0.046 |
| RM + 10% WMP | 0.66                             | 0.031 |
| RM + 15% WMP | 0.77                             | 0.021 |
| RM + 20% WMP | 0.84                             | 0.026 |
| RM + 30% WMP | 1.05                             | 0.095 |
| RM + 40% WMP | 1.37                             | 0.057 |
| RM + 50% WMP | 1.69                             | 0.089 |
| RM + 60% WMP | 1.85                             | 0.076 |
| RM + 70% WMP | 2.02                             | 0.113 |
| RM + 80% WMP | 2.22                             | 0.106 |
| RM + 90% WMP | 2.58                             | 0.095 |
| 100% WMP     | 2.95                             | 0.112 |

\* % WMP = percentage of reconstituted whole milk powder

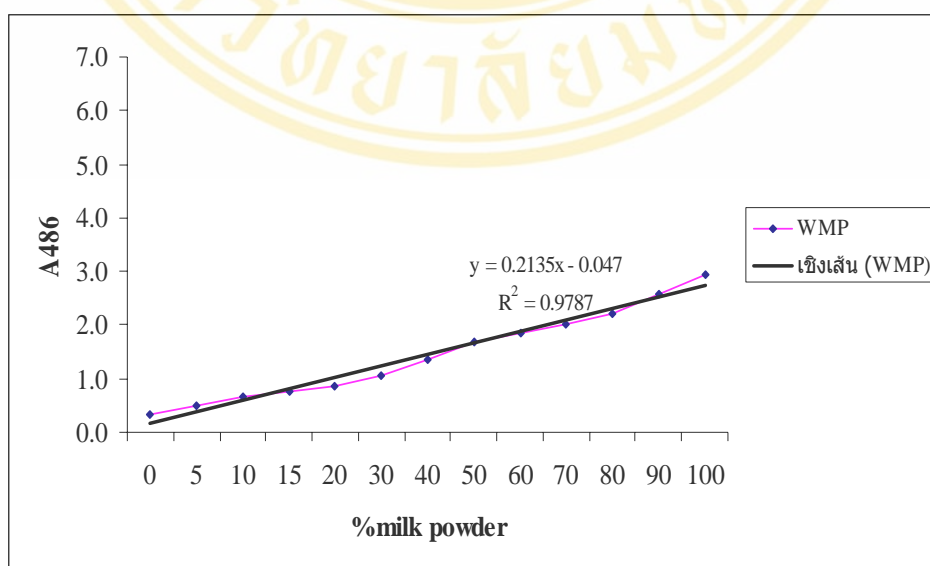


Figure 12 The absorbance of deoxyketone content in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder.

Table 14 The absorbance of deoxyketone content in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

| % SMP*      | Average absorbance at 486 nm. | SD    |
|-------------|-------------------------------|-------|
| 100% RM     | 0.36                          | 0.033 |
| RM + 5%SMP  | 0.67                          | 0.076 |
| RM + 10%SMP | 0.87                          | 0.044 |
| RM + 15%SMP | 0.90                          | 0.037 |
| RM + 20%SMP | 1.29                          | 0.083 |
| RM + 30%SMP | 1.71                          | 0.142 |
| RM + 40%SMP | 2.21                          | 0.115 |
| RM + 50%SMP | 2.64                          | 0.153 |
| RM + 60%SMP | 3.06                          | 0.197 |
| RM + 70%SMP | 3.27                          | 0.215 |
| RM + 80%SMP | 3.89                          | 0.181 |
| RM + 90%SMP | 4.27                          | 0.347 |
| 100% SMP    | 6.18                          | 0.791 |

\* %SMP = percentage of reconstituted skimmed milk powder

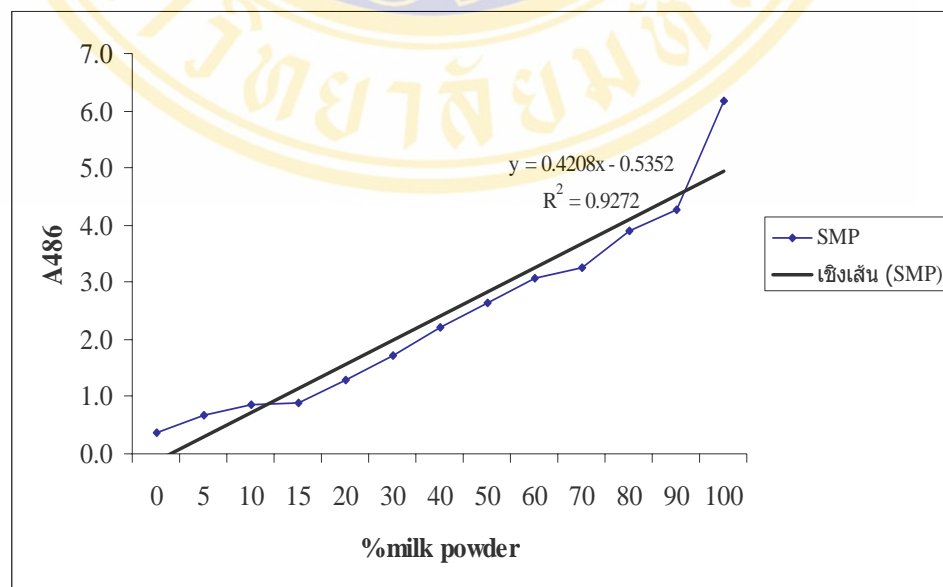


Figure 13 The absorbance of deoxyketone content in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

Table 15 The absorbance of deoxyketone content in pasteurized milk samples purchased from supermarket in Bangkok.

| Company             | Average absorbance<br>at 486 nm. | SD    |
|---------------------|----------------------------------|-------|
| <b>Whole milk</b>   |                                  |       |
| A                   | 1.329                            | 0.163 |
| B                   | 1.273                            | 0.121 |
| C                   | 1.519                            | 0.272 |
| D                   | 1.238                            | 0.240 |
| E                   | 1.354                            | 0.241 |
| <b>Low-fat milk</b> |                                  |       |
| A                   | 1.826                            | 0.192 |
| B                   | 1.127                            | 0.021 |
| C                   | 2.027                            | 0.376 |
| D                   | 0.927                            | 0.114 |
| E                   | 0.810                            | 0.076 |

Table 16 The absorbance of deoxyketone content in UHT milk samples purchased from supermarket in Bangkok.

| Company             | Average absorbance<br>at 486 nm. | SD    |
|---------------------|----------------------------------|-------|
| <b>Whole milk</b>   |                                  |       |
| A                   | 2.924                            | 0.178 |
| B                   | 3.354                            | 0.280 |
| C                   | 2.365                            | 0.213 |
| D                   | 3.800                            | 0.094 |
| E                   | 2.602                            | 0.048 |
| F                   | 1.902                            | 0.090 |
| G                   | 2.818                            | 0.254 |
| <b>Low-fat milk</b> |                                  |       |
| A                   | 3.121                            | 0.240 |
| B                   | 2.382                            | 0.438 |

## 4.2 Biochemical analysis of milk samples

All of milk samples were subjected to biochemical analysis using Milkoscan™ 4000. The contents of milk compositions including fat, protein, lactose, urea, citric acid, solid not fat (SNF), and total solid (TS) in raw milk combined with reconstituted whole milk powder and those in raw milk combined with reconstituted skimmed milk powder were presented in Table 17 and Table 18, respectively.

The results showed that all of the components in samples prepared by combining raw milk with reconstituted whole milk powder were slightly increased when the level of whole milk powder was higher. The results obtained from the biochemical analysis of the samples prepared by combining raw milk with reconstituted skimmed milk powder were in the same manner. An exception was that fat content tended to decrease with higher level of skimmed milk powder. The levels of compositions in pasteurized milk and UHT milk purchased from supermarkets in Bangkok were shown in Table 19 and Table 20, respectively.

Table 17 Biochemical analysis of samples prepared by combining raw milk with various amounts of reconstituted whole milk powder

| %WMP*       | Content (g/100 ml)<br>Mean $\pm$ SD |                  |                  |                   |                   |                   |                  |  |  |  |
|-------------|-------------------------------------|------------------|------------------|-------------------|-------------------|-------------------|------------------|--|--|--|
|             | Proximate analysis                  |                  |                  |                   |                   | Chemical analysis |                  |  |  |  |
|             | Fat                                 | Protein          | Lactose          | SNF               | TS                | Urea              | Cit.Ac           |  |  |  |
| 100% RM     | 4.31 $\pm$ 0.226                    | 3.12 $\pm$ 0.007 | 4.56 $\pm$ 0.049 | 8.37 $\pm$ 0.049  | 12.68 $\pm$ 0.276 | 0.02 $\pm$ 0.011  | 0.14 $\pm$ 0.003 |  |  |  |
| RM + 5%WMP  | 4.26 $\pm$ 0.233                    | 3.14 $\pm$ 0.021 | 4.60 $\pm$ 0.049 | 8.43 $\pm$ 0.057  | 12.39 $\pm$ 0.134 | 0.03 $\pm$ 0.012  | 0.14 $\pm$ 0.004 |  |  |  |
| RM + 10%WMP | 4.25 $\pm$ 0.226                    | 3.17 $\pm$ 0.021 | 4.67 $\pm$ 0.035 | 8.54 $\pm$ 0.049  | 12.50 $\pm$ 0.127 | 0.03 $\pm$ 0.010  | 0.14 $\pm$ 0.004 |  |  |  |
| RM + 15%WMP | 4.27 $\pm$ 0.205                    | 3.20 $\pm$ 0.028 | 4.74 $\pm$ 0.028 | 8.64 $\pm$ 0.057  | 12.65 $\pm$ 0.106 | 0.03 $\pm$ 0.012  | 0.14 $\pm$ 0.004 |  |  |  |
| RM + 20%WMP | 4.26 $\pm$ 0.205                    | 3.23 $\pm$ 0.035 | 4.83 $\pm$ 0.042 | 8.76 $\pm$ 0.078  | 12.76 $\pm$ 0.071 | 0.03 $\pm$ 0.012  | 0.14 $\pm$ 0.004 |  |  |  |
| RM + 30%WMP | 4.25 $\pm$ 0.198                    | 3.25 $\pm$ 0.007 | 4.97 $\pm$ 0.021 | 8.90 $\pm$ 0.028  | 13.15 $\pm$ 0.226 | 0.03 $\pm$ 0.012  | 0.15 $\pm$ 0.004 |  |  |  |
| RM + 40%WMP | 4.22 $\pm$ 0.163                    | 3.26 $\pm$ 0.000 | 5.11 $\pm$ 0.000 | 9.07 $\pm$ 0.000  | 13.29 $\pm$ 0.163 | 0.04 $\pm$ 0.006  | 0.15 $\pm$ 0.002 |  |  |  |
| RM + 50%WMP | 4.21 $\pm$ 0.148                    | 3.28 $\pm$ 0.000 | 5.28 $\pm$ 0.007 | 9.26 $\pm$ 0.007  | 13.46 $\pm$ 0.134 | 0.04 $\pm$ 0.006  | 0.15 $\pm$ 0.006 |  |  |  |
| RM + 60%WMP | 4.17 $\pm$ 0.099                    | 3.30 $\pm$ 0.007 | 5.42 $\pm$ 0.014 | 9.41 $\pm$ 0.014  | 13.58 $\pm$ 0.085 | 0.04 $\pm$ 0.004  | 0.15 $\pm$ 0.001 |  |  |  |
| RM + 70%WMP | 4.15 $\pm$ 0.071                    | 3.30 $\pm$ 0.007 | 5.57 $\pm$ 0.000 | 9.57 $\pm$ 0.007  | 13.72 $\pm$ 0.078 | 0.04 $\pm$ 0.001  | 0.16 $\pm$ 0.002 |  |  |  |
| RM + 80%WMP | 4.13 $\pm$ 0.057                    | 3.31 $\pm$ 0.014 | 5.74 $\pm$ 0.007 | 9.75 $\pm$ 0.007  | 13.88 $\pm$ 0.064 | 0.05 $\pm$ 0.001  | 0.16 $\pm$ 0.002 |  |  |  |
| RM + 90%WMP | 4.10 $\pm$ 0.028                    | 3.33 $\pm$ 0.014 | 5.92 $\pm$ 0.000 | 9.95 $\pm$ 0.000  | 14.05 $\pm$ 0.042 | 0.05 $\pm$ 0.002  | 0.16 $\pm$ 0.001 |  |  |  |
| 100% WMP    | 4.10 $\pm$ 0.021                    | 3.36 $\pm$ 0.021 | 6.08 $\pm$ 0.007 | 10.13 $\pm$ 0.021 | 14.22 $\pm$ 0.049 | 0.06 $\pm$ 0.001  | 0.16 $\pm$ 0.001 |  |  |  |

\* % WMP = percentage of reconstituted whole milk powder

Table 18 Biochemical analysis of samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder

| %SMP*       | Content (g/100 ml)<br>Mean ± SD |              |              |               |               |                   |              |  |  |  |
|-------------|---------------------------------|--------------|--------------|---------------|---------------|-------------------|--------------|--|--|--|
|             | Proximate analysis              |              |              |               |               | Chemical analysis |              |  |  |  |
|             | Fat                             | Protein      | Lactose      | SNF           | TS            | Urea              | Cit.Ac       |  |  |  |
| 100% RM     | 4.31 ± 0.226                    | 3.12 ± 0.007 | 4.56 ± 0.049 | 8.37 ± 0.049  | 12.68 ± 0.276 | 0.02 ± 0.011      | 0.14 ± 0.003 |  |  |  |
| RM + 5%SMP  | 4.14 ± 0.191                    | 3.19 ± 0.000 | 4.68 ± 0.035 | 8.57 ± 0.035  | 12.70 ± 0.226 | 0.03 ± 0.008      | 0.14 ± 0.003 |  |  |  |
| RM + 10%SMP | 3.93 ± 0.205                    | 3.27 ± 0.007 | 4.81 ± 0.000 | 8.78 ± 0.007  | 12.71 ± 0.205 | 0.04 ± 0.008      | 0.15 ± 0.004 |  |  |  |
| RM + 15%SMP | 3.74 ± 0.205                    | 3.34 ± 0.014 | 4.96 ± 0.007 | 9.00 ± 0.007  | 12.73 ± 0.219 | 0.04 ± 0.006      | 0.15 ± 0.008 |  |  |  |
| RM + 20%SMP | 3.53 ± 0.205                    | 3.42 ± 0.007 | 5.10 ± 0.014 | 9.22 ± 0.007  | 12.74 ± 0.198 | 0.05 ± 0.008      | 0.15 ± 0.002 |  |  |  |
| RM + 30%SMP | 3.11 ± 0.205                    | 3.57 ± 0.021 | 5.40 ± 0.035 | 9.66 ± 0.007  | 12.76 ± 0.198 | 0.07 ± 0.008      | 0.16 ± 0.004 |  |  |  |
| RM + 40%SMP | 2.71 ± 0.156                    | 3.70 ± 0.014 | 5.69 ± 0.021 | 10.09 ± 0.021 | 12.79 ± 0.141 | 0.08 ± 0.006      | 0.17 ± 0.002 |  |  |  |
| RM + 50%SMP | 2.31 ± 0.120                    | 3.84 ± 0.021 | 5.99 ± 0.000 | 10.53 ± 0.021 | 12.84 ± 0.134 | 0.09 ± 0.002      | 0.18 ± 0.002 |  |  |  |
| RM + 60%SMP | 1.90 ± 0.092                    | 3.98 ± 0.014 | 6.28 ± 0.014 | 10.96 ± 0.000 | 12.86 ± 0.085 | 0.11 ± 0.006      | 0.19 ± 0.001 |  |  |  |
| RM + 70%SMP | 1.49 ± 0.078                    | 4.11 ± 0.014 | 6.59 ± 0.035 | 11.39 ± 0.042 | 12.88 ± 0.042 | 0.12 ± 0.002      | 0.19 ± 0.001 |  |  |  |
| RM + 80%SMP | 1.09 ± 0.049                    | 4.25 ± 0.007 | 6.87 ± 0.042 | 11.82 ± 0.049 | 12.90 ± 0.000 | 0.14 ± 0.001      | 0.20 ± 0.001 |  |  |  |
| RM + 90%SMP | 0.66 ± 0.028                    | 4.38 ± 0.007 | 7.23 ± 0.021 | 12.30 ± 0.028 | 12.96 ± 0.000 | 0.15 ± 0.000      | 0.21 ± 0.001 |  |  |  |
| 100% SMP    | 0.26 ± 0.014                    | 4.52 ± 0.007 | 7.54 ± 0.028 | 12.76 ± 0.035 | 13.02 ± 0.021 | 0.17 ± 0.002      | 0.22 ± 0.001 |  |  |  |

\* %SMP = percentage of reconstituted skimmed milk powder

Table 19 Biochemical analysis of pasteurized milk samples purchased from supermarkets in Bangkok

| Company             | Content (g/100 ml)<br>Mean $\pm$ SD |                  |                  |                   |                   |                   |                  |  |  |  |  |  |  |
|---------------------|-------------------------------------|------------------|------------------|-------------------|-------------------|-------------------|------------------|--|--|--|--|--|--|
|                     | Proximate analysis                  |                  |                  |                   |                   | Chemical analysis |                  |  |  |  |  |  |  |
|                     | Fat                                 | Protein          | Lactose          | SNF               | TS                | Urea              | Cit.Ac           |  |  |  |  |  |  |
| <b>Whole milk</b>   |                                     |                  |                  |                   |                   |                   |                  |  |  |  |  |  |  |
| A                   | 3.60 $\pm$ 0.007                    | 3.14 $\pm$ 0.021 | 4.71 $\pm$ 0.014 | 8.54 $\pm$ 0.028  | 12.14 $\pm$ 0.035 | 0.03 $\pm$ 0.001  | 0.15 $\pm$ 0.002 |  |  |  |  |  |  |
| B                   | 3.93 $\pm$ 0.000                    | 3.13 $\pm$ 0.007 | 4.72 $\pm$ 0.014 | 8.55 $\pm$ 0.021  | 12.47 $\pm$ 0.014 | 0.03 $\pm$ 0.004  | 0.14 $\pm$ 0.002 |  |  |  |  |  |  |
| C                   | 3.96 $\pm$ 0.000                    | 3.29 $\pm$ 0.023 | 5.27 $\pm$ 0.028 | 9.26 $\pm$ 0.042  | 13.22 $\pm$ 0.042 | 0.04 $\pm$ 0.001  | 0.15 $\pm$ 0.000 |  |  |  |  |  |  |
| D                   | 3.68 $\pm$ 0.007                    | 3.04 $\pm$ 0.007 | 4.56 $\pm$ 0.000 | 8.30 $\pm$ 0.007  | 11.97 $\pm$ 0.014 | 0.04 $\pm$ 0.004  | 0.15 $\pm$ 0.001 |  |  |  |  |  |  |
| E                   | 3.35 $\pm$ 0.007                    | 3.24 $\pm$ 0.014 | 4.83 $\pm$ 0.007 | 8.77 $\pm$ 0.014  | 12.11 $\pm$ 0.028 | 0.04 $\pm$ 0.001  | 0.16 $\pm$ 0.000 |  |  |  |  |  |  |
| <b>Low-fat milk</b> |                                     |                  |                  |                   |                   |                   |                  |  |  |  |  |  |  |
| A                   | 1.67 $\pm$ 0.007                    | 3.15 $\pm$ 0.021 | 4.76 $\pm$ 0.021 | 8.61 $\pm$ 0.035  | 10.27 $\pm$ 0.042 | 0.06 $\pm$ 0.001  | 0.18 $\pm$ 0.001 |  |  |  |  |  |  |
| B                   | 0.55 $\pm$ 0.007                    | 3.22 $\pm$ 0.007 | 6.33 $\pm$ 0.014 | 10.25 $\pm$ 0.021 | 10.80 $\pm$ 0.035 | 0.09 $\pm$ 0.001  | 0.23 $\pm$ 0.002 |  |  |  |  |  |  |
| C                   | 0.97 $\pm$ 0.007                    | 3.68 $\pm$ 0.014 | 6.12 $\pm$ 0.014 | 10.50 $\pm$ 0.028 | 11.47 $\pm$ 0.028 | 0.09 $\pm$ 0.002  | 0.21 $\pm$ 0.002 |  |  |  |  |  |  |
| D                   | 2.20 $\pm$ 0.007                    | 3.14 $\pm$ 0.007 | 4.73 $\pm$ 0.014 | 8.57 $\pm$ 0.028  | 10.77 $\pm$ 0.035 | 0.06 $\pm$ 0.004  | 0.18 $\pm$ 0.004 |  |  |  |  |  |  |
| E                   | 1.64 $\pm$ 0.007                    | 3.32 $\pm$ 0.021 | 4.96 $\pm$ 0.014 | 8.98 $\pm$ 0.035  | 10.60 $\pm$ 0.042 | 0.08 $\pm$ 0.001  | 0.19 $\pm$ 0.001 |  |  |  |  |  |  |

Table 20 Biochemical analysis of UHT milk samples purchased from supermarkets in Bangkok

| Company              | Content (g/100 ml)<br>Mean ± SD |              |              |               |               |                   |              |  |  |  |
|----------------------|---------------------------------|--------------|--------------|---------------|---------------|-------------------|--------------|--|--|--|
|                      | Proximate analysis              |              |              |               |               | Chemical analysis |              |  |  |  |
|                      | Fat                             | Protein      | Lactose      | SNF           | TS            | Urea              | Cit.Ac       |  |  |  |
| <b>Whole milk</b>    |                                 |              |              |               |               |                   |              |  |  |  |
| A                    | 3.65 ± 0.000                    | 2.87 ± 0.014 | 4.67 ± 0.007 | 8.24 ± 0.021  | 11.89 ± 0.021 | 0.05 ± 0.004      | 0.15 ± 0.001 |  |  |  |
| B                    | 3.79 ± 0.007                    | 3.16 ± 0.007 | 4.65 ± 0.000 | 8.51 ± 0.007  | 12.29 ± 0.014 | 0.04 ± 0.004      | 0.15 ± 0.000 |  |  |  |
| C                    | 4.13 ± 0.000                    | 3.21 ± 0.014 | 4.78 ± 0.007 | 8.68 ± 0.021  | 12.81 ± 0.028 | 0.04 ± 0.002      | 0.15 ± 0.000 |  |  |  |
| D                    | 4.53 ± 0.014                    | 3.29 ± 0.007 | 4.51 ± 0.007 | 8.49 ± 0.014  | 13.02 ± 0.021 | 0.04 ± 0.004      | 0.14 ± 0.002 |  |  |  |
| E                    | 3.79 ± 0.007                    | 3.12 ± 0.007 | 4.58 ± 0.000 | 8.40 ± 0.007  | 12.18 ± 0.014 | 0.05 ± 0.004      | 0.14 ± 0.001 |  |  |  |
| F                    | 3.96 ± 0.007                    | 3.13 ± 0.021 | 4.57 ± 0.007 | 8.40 ± 0.021  | 12.35 ± 0.035 | 0.04 ± 0.002      | 0.16 ± 0.004 |  |  |  |
| G                    | 3.94 ± 0.007                    | 3.15 ± 0.014 | 4.54 ± 0.000 | 8.40 ± 0.007  | 12.33 ± 0.021 | 0.04 ± 0.001      | 0.15 ± 0.001 |  |  |  |
| <b>Low- fat milk</b> |                                 |              |              |               |               |                   |              |  |  |  |
| A                    | 1.47 ± 0.000                    | 3.44 ± 0.014 | 5.60 ± 0.014 | 9.74 ± 0.035  | 11.21 ± 0.028 | 0.10 ± 0.000      | 0.19 ± 0.004 |  |  |  |
| B                    | 1.37 ± 0.007                    | 3.66 ± 0.010 | 5.76 ± 0.007 | 10.12 ± 0.021 | 11.48 ± 0.028 | 0.09 ± 0.003      | 0.19 ± 0.001 |  |  |  |

### 4.3 Determination of the ratios of milk composition in milk samples

The results obtained from the biochemical analysis were used to calculate for 42 ratios of milk compositions. However, there were only six ratios that can be used to differentiate between fresh milk and combined milk. The six ratios selected were Fat/Urea, Fat/Cit.Ac, Protein/Urea, Lactose/Urea, SNF/Urea, and TS/Urea. The results were shown in Table 21 for samples prepared by combining raw milk with various amounts of reconstituted whole milk powder and Table 22 for samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder, respectively. Figure 14 to Figure 25 represented the selected ratios of milk composition in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder and those prepared by combining raw milk with reconstituted skimmed milk powder. The regression coefficients ( $R^2$ ) and linear regression equations for the relationships for each type of milk samples were also shown. Table 23 and Table 24 showed the ratios of milk composition in market fresh milk (pasteurized milk and UHT milk) purchased from supermarkets in Bangkok.

The results showed that all of the ratios of milk composition in samples prepared by combining raw milk with reconstituted whole milk powder were obviously decreased when the level of whole milk powder was higher. The results obtained from the ratios of milk composition of the samples prepared by combining raw milk with reconstituted skimmed milk powder were in the same manner.

Table 21 Ratios of milk composition in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder

| <b>%WMP*</b> | <b>Fat/Urea</b> | <b>Fat/Cit.Ac</b> | <b>Protein/Urea</b> | <b>Lact./Urea</b> | <b>SNF/Urea</b> | <b>TS/Urea</b> |
|--------------|-----------------|-------------------|---------------------|-------------------|-----------------|----------------|
| 100% RM      | 189.01          | 30.89             | 135.26              | 198.13            | 363.49          | 552.50         |
| RM + 5% WMP  | 173.12          | 30.06             | 126.23              | 185.18            | 339.42          | 496.75         |
| RM + 10% WMP | 170.40          | 29.81             | 125.82              | 185.49            | 339.25          | 495.40         |
| RM + 15% WMP | 165.93          | 29.82             | 123.47              | 182.78            | 333.22          | 486.14         |
| RM + 20% WMP | 152.69          | 29.54             | 114.89              | 171.99            | 311.77          | 453.09         |
| RM + 30% WMP | 136.73          | 28.90             | 103.57              | 158.53            | 284.11          | 420.84         |
| RM + 40% WMP | 122.40          | 28.19             | 94.38               | 147.93            | 262.57          | 384.98         |
| RM + 50% WMP | 102.81          | 27.85             | 79.98               | 128.61            | 225.65          | 328.33         |
| RM + 60% WMP | 106.11          | 27.08             | 83.75               | 137.75            | 239.17          | 345.28         |
| RM + 70% WMP | 95.43           | 26.52             | 75.76               | 128.06            | 219.92          | 315.34         |
| RM + 80% WMP | 89.84           | 26.22             | 72.00               | 124.73            | 211.95          | 301.79         |
| RM + 90% WMP | 84.60           | 25.79             | 68.72               | 122.18            | 205.35          | 289.95         |
| 100% WMP     | 74.48           | 25.36             | 61.03               | 110.49            | 184.16          | 258.55         |

\* % WMP = percentage of reconstituted whole milk powder

Table 22 Ratios of milk composition in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder

| % SMP*      | Fat/Urea | Fat/Cit.Ac | Protein/Urea | Lact./Urea | SNF/Urea | TS/Urea |
|-------------|----------|------------|--------------|------------|----------|---------|
| 100% RM     | 196.31   | 31.45      | 140.36       | 205.63     | 377.22   | 573.53  |
| RM + 5%SMP  | 136.17   | 28.91      | 104.45       | 153.22     | 280.60   | 416.77  |
| RM + 10%SMP | 106.55   | 26.78      | 88.14        | 129.82     | 236.85   | 343.51  |
| RM + 15%SMP | 85.90    | 24.98      | 76.56        | 113.54     | 206.15   | 291.94  |
| RM + 20%SMP | 70.43    | 22.96      | 67.91        | 101.38     | 183.21   | 253.64  |
| RM + 30%SMP | 48.39    | 18.98      | 55.34        | 83.68      | 149.81   | 198.20  |
| RM + 40%SMP | 34.28    | 15.89      | 46.70        | 71.73      | 127.25   | 161.47  |
| RM + 50%SMP | 24.67    | 13.06      | 41.03        | 64.08      | 112.60   | 137.32  |
| RM + 60%SMP | 18.02    | 10.16      | 37.80        | 59.63      | 104.08   | 122.14  |
| RM + 70%SMP | 12.13    | 7.66       | 33.56        | 53.76      | 92.99    | 105.16  |
| RM + 80%SMP | 7.95     | 5.32       | 31.10        | 50.33      | 86.56    | 94.51   |
| RM + 90%SMP | 4.43     | 3.17       | 29.36        | 48.49      | 82.55    | 86.98   |
| 100% SMP    | 1.55     | 1.21       | 26.96        | 45.02      | 76.16    | 77.71   |

\* %SMP = percentage of reconstituted skimmed milk powder

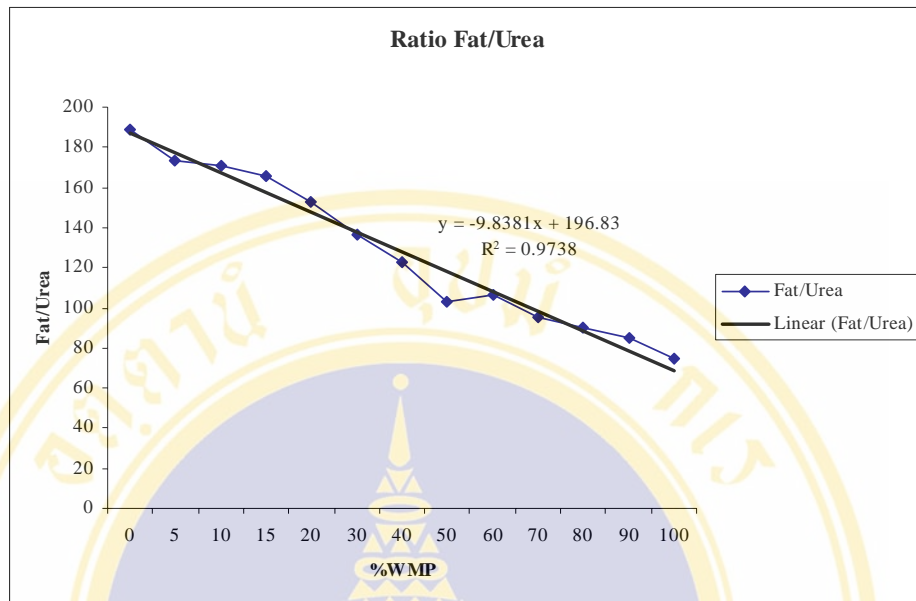


Figure 14 Fat/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder.

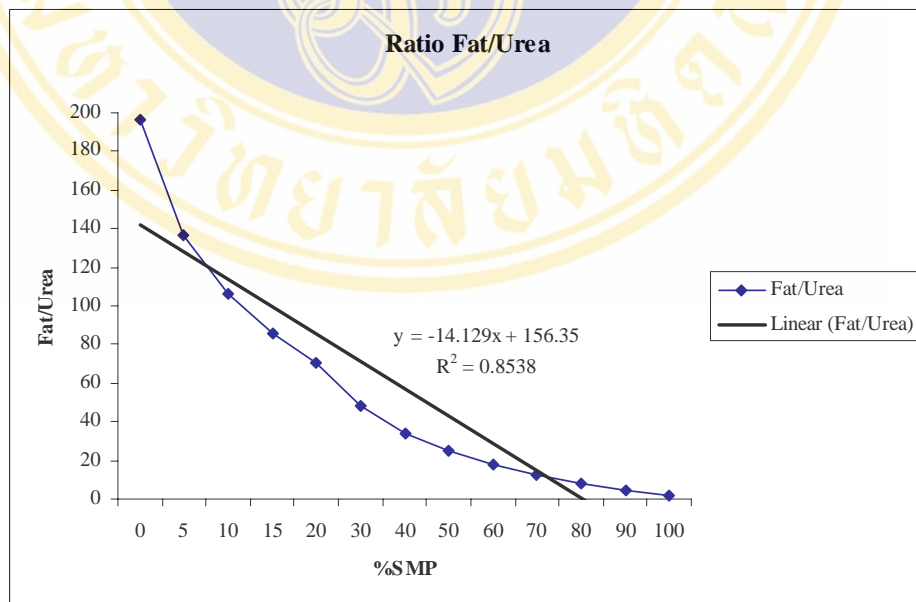


Figure 15 Fat/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

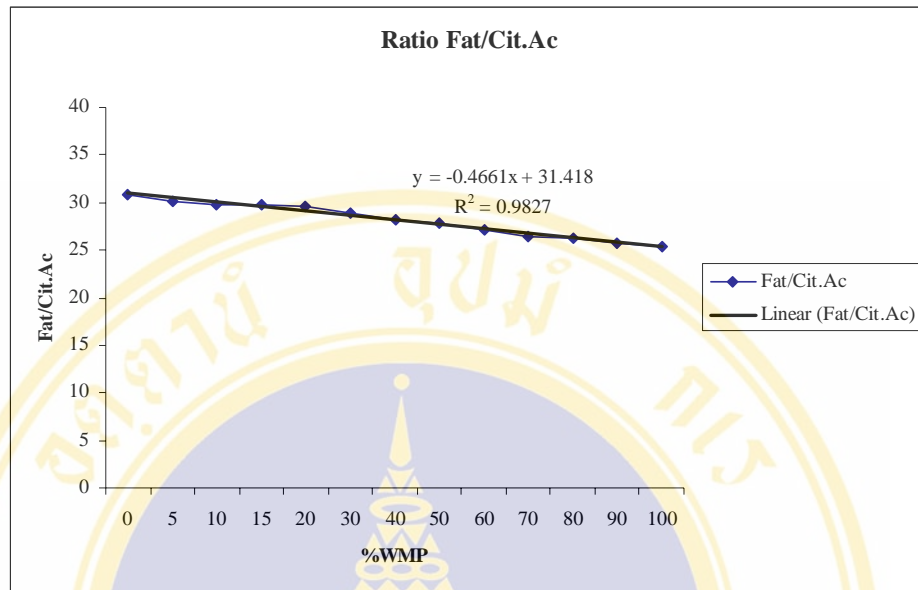


Figure 16 Fat/Cit.Ac ratios in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder.

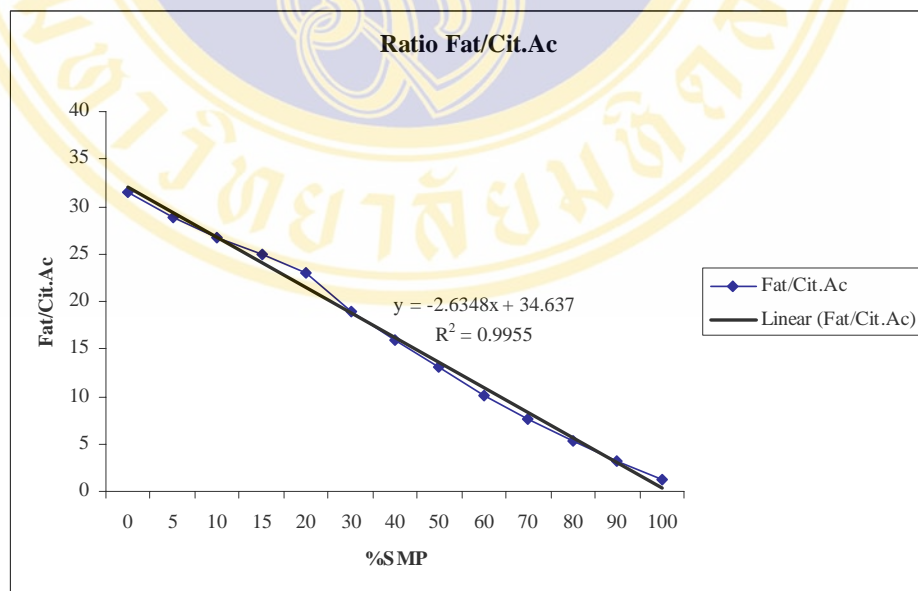


Figure 17 Fat/Cit.Ac ratios in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

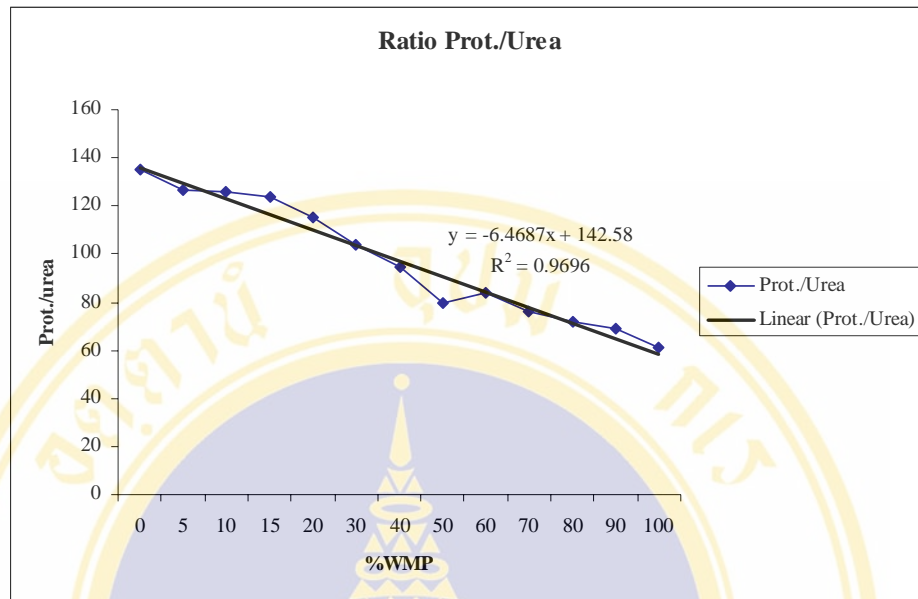


Figure 18 Protein/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder.

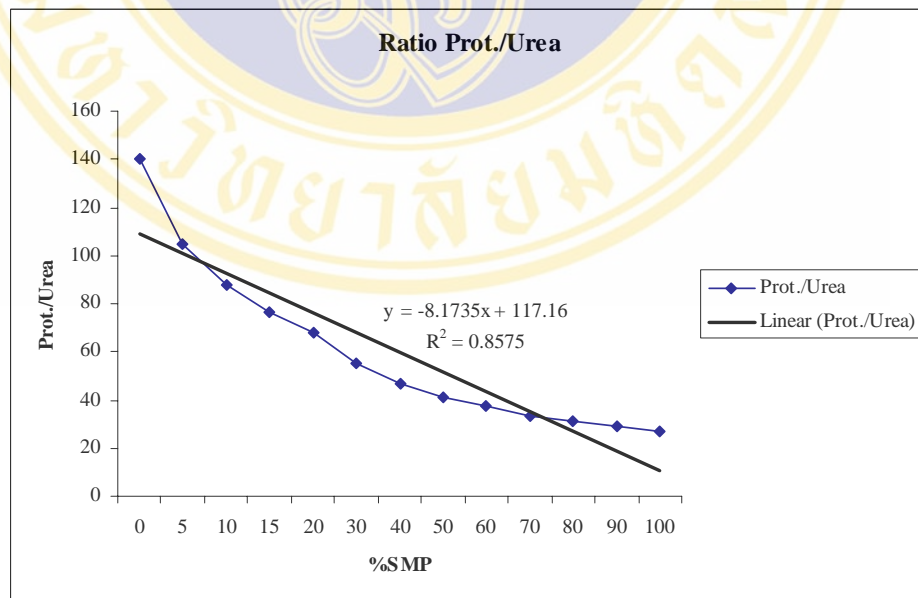


Figure 19 Protein/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

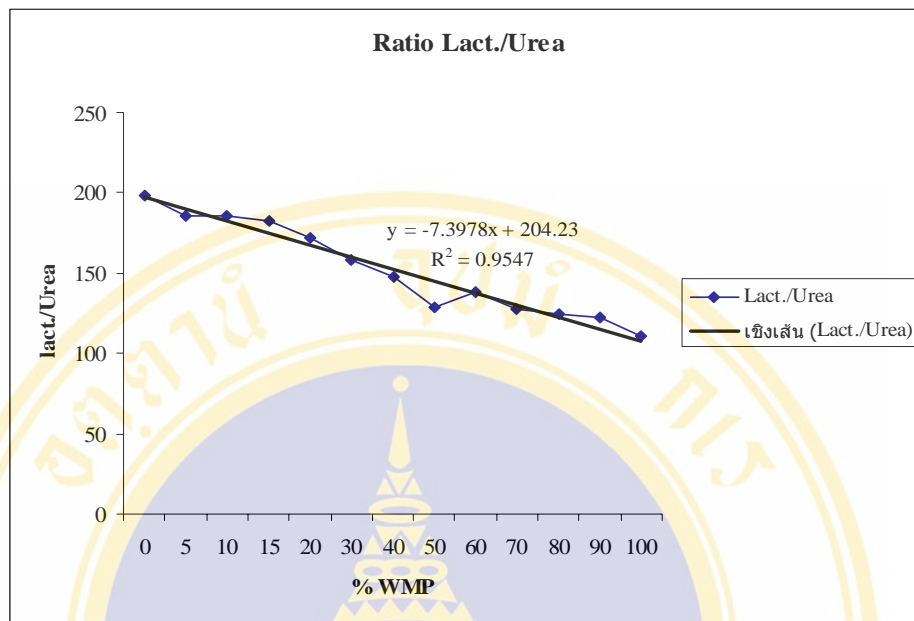


Figure 20 Lactose/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder.

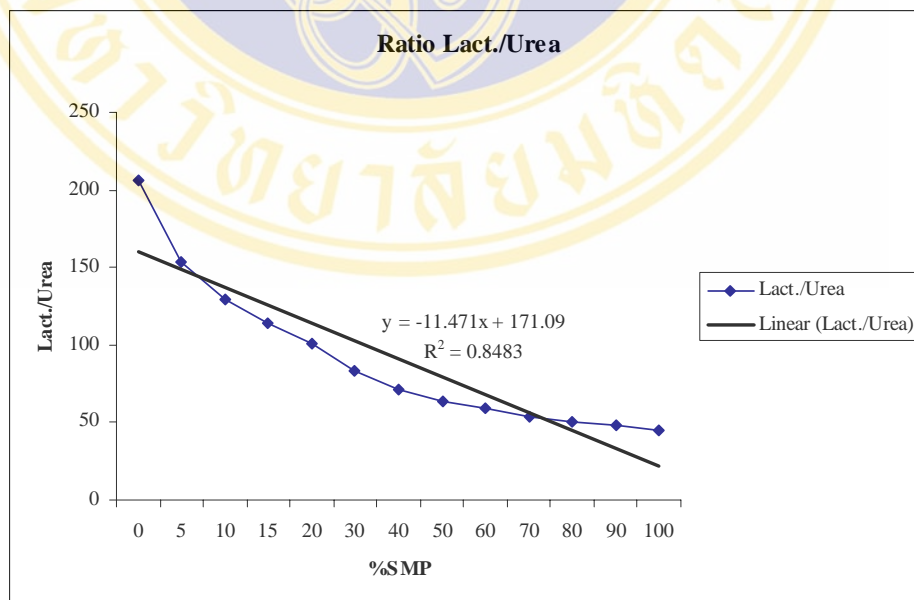


Figure 21 Lactose/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

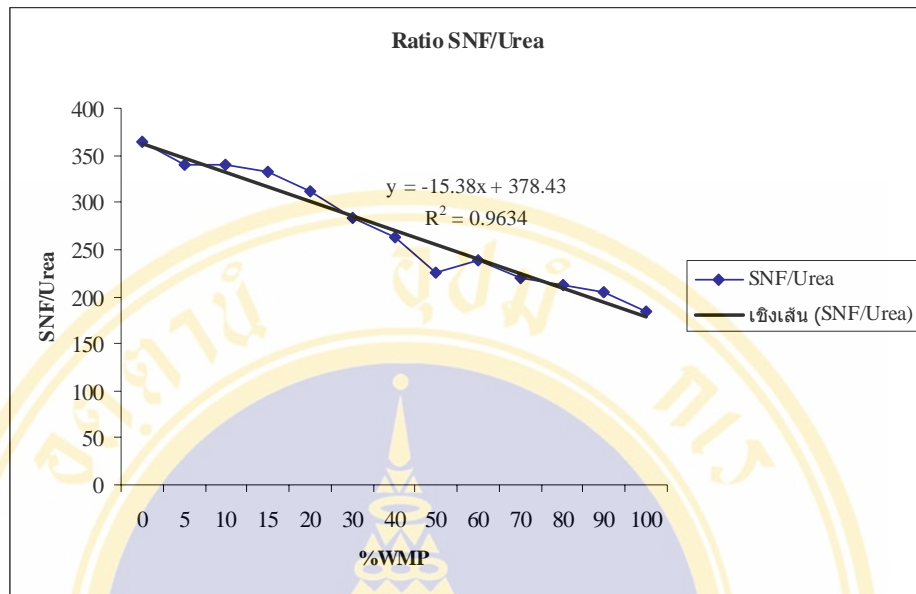


Figure 22 SNF/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder.

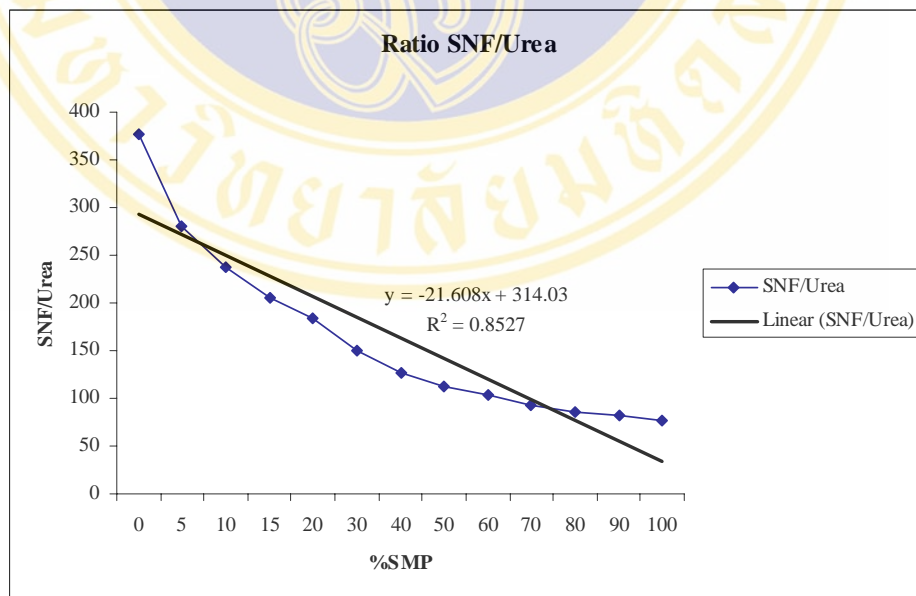


Figure 23 SNF/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

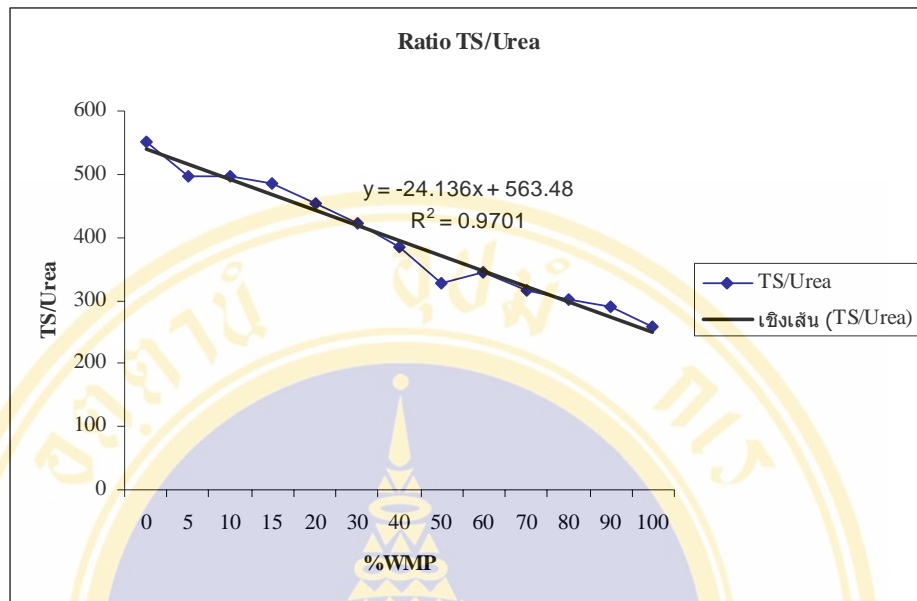


Figure 24 TS/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder.

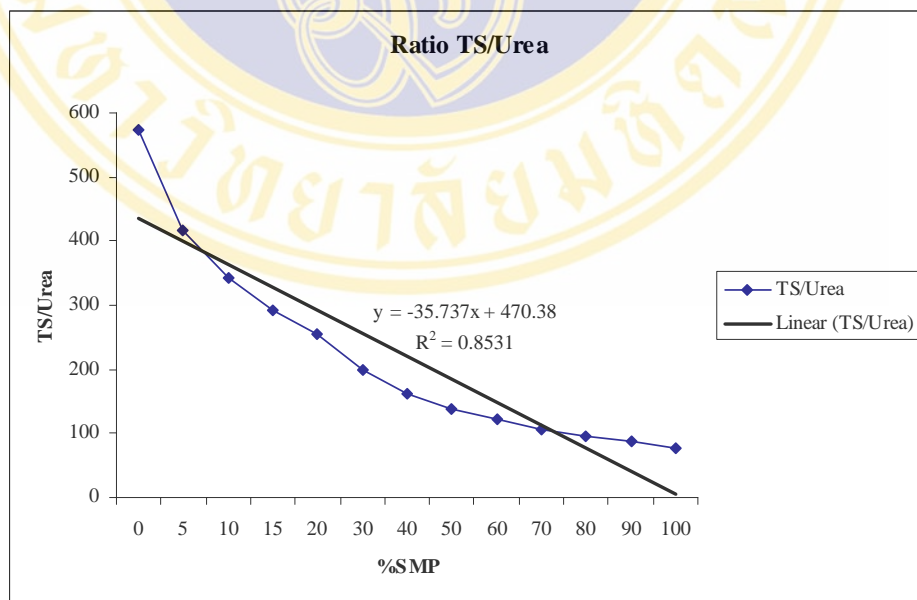


Figure 25 TS/Urea ratios in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

Table 23 Ratios of milk composition in pasteurized milk purchased from supermarkets in Bangkok.

| Company             | Fat/Urea | Fat/Cit.Ac | Protein/Urea | Lact./Urea | SNF/Urea | TS/Urea |
|---------------------|----------|------------|--------------|------------|----------|---------|
| <b>Whole milk</b>   |          |            |              |            |          |         |
| A                   | 110.64   | 23.73      | 96.48        | 144.95     | 262.82   | 373.46  |
| B                   | 117.97   | 27.39      | 93.79        | 141.66     | 256.47   | 374.30  |
| C                   | 101.61   | 26.40      | 84.42        | 135.23     | 237.61   | 339.22  |
| D                   | 95.85    | 23.79      | 79.16        | 118.94     | 216.36   | 312.21  |
| E                   | 84.70    | 20.78      | 82.04        | 122.17     | 222.06   | 306.63  |
| <b>Low-fat milk</b> |          |            |              |            |          |         |
| A                   | 26.02    | 9.15       | 49.16        | 74.32      | 134.49   | 160.52  |
| B                   | 6.12     | 2.37       | 36.13        | 71.13      | 115.13   | 121.30  |
| C                   | 10.32    | 4.63       | 39.37        | 65.47      | 112.33   | 122.71  |
| D                   | 38.24    | 12.37      | 54.62        | 82.41      | 149.31   | 187.55  |
| E                   | 21.66    | 8.84       | 43.91        | 65.70      | 118.88   | 140.41  |

Table 24 Ratios of milk composition in UHT milk purchased from supermarkets in Bangkok.

| Company             | Fat/Urea | Fat/Cit.Ac | Protein/Urea | Lact./Urea | SNF/Urea | TS/Urea |
|---------------------|----------|------------|--------------|------------|----------|---------|
| <b>Whole milk</b>   |          |            |              |            |          |         |
| A                   | 69.68    | 24.09      | 54.78        | 89.05      | 157.20   | 226.88  |
| B                   | 101.38   | 24.74      | 84.50        | 124.55     | 227.80   | 329.18  |
| C                   | 116.55   | 28.10      | 90.60        | 134.75     | 244.82   | 361.51  |
| D                   | 112.26   | 32.02      | 81.41        | 111.65     | 210.42   | 322.56  |
| E                   | 76.66    | 26.94      | 63.09        | 92.76      | 170.02   | 246.68  |
| F                   | 97.78    | 24.50      | 77.25        | 112.87     | 207.55   | 305.21  |
| G                   | 89.48    | 25.89      | 71.62        | 103.24     | 190.89   | 280.25  |
| <b>Low-fat milk</b> |          |            |              |            |          |         |
| A                   | 14.70    | 7.84       | 34.40        | 56.00      | 97.35    | 112.10  |
| B                   | 14.68    | 7.38       | 39.37        | 61.91      | 108.81   | 123.49  |

#### 4.4 Determination of milk fat particle size in milk samples

The milk fat particle size in sample prepared by combining raw milk with various amounts of reconstituted whole milk powder was shown in Table 25. The milk fat particle size in raw milk was 4.372  $\mu\text{m}$ . whereas these in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder (5-100%) ranged from 4.312 to 0.253  $\mu\text{m}$ .

Table 26 showed the milk fat particle size in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder. The results indicated that the milk fat particle size sharply decreased when more than 30% of reconstituted skimmed milk powder was added. Figure 26 and Figure 27 showed the regression coefficients ( $R^2$ ) and linear regression equations of the relationships between the milk fat particle size and the amounts of added reconstituted milk powder. For the milk fat particle size in pasteurized milk and UHT milk were shown in Table 27 and Table 28, respectively.

#### 4.4.1 Milk fat particle size in samples prepared by combining raw milk with various amount of reconstituted whole milk powder.

Table 25 Milk fat particle size in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder

| % WMP*       | Milk fat particle size (µm) |
|--------------|-----------------------------|
| 100% RM      | 4.372                       |
| RM + 5% WMP  | 4.312                       |
| RM + 10% WMP | 4.229                       |
| RM + 15% WMP | 4.118                       |
| RM + 20% WMP | 3.854                       |
| RM + 30% WMP | 3.099                       |
| RM + 40% WMP | 1.241                       |
| RM + 50% WMP | 0.674                       |
| RM + 60% WMP | 0.478                       |
| RM + 70% WMP | 0.382                       |
| RM + 80% WMP | 0.330                       |
| RM + 90% WMP | 0.284                       |
| 100% WMP     | 0.253                       |

\* % WMP = percentage of reconstituted whole milk powder

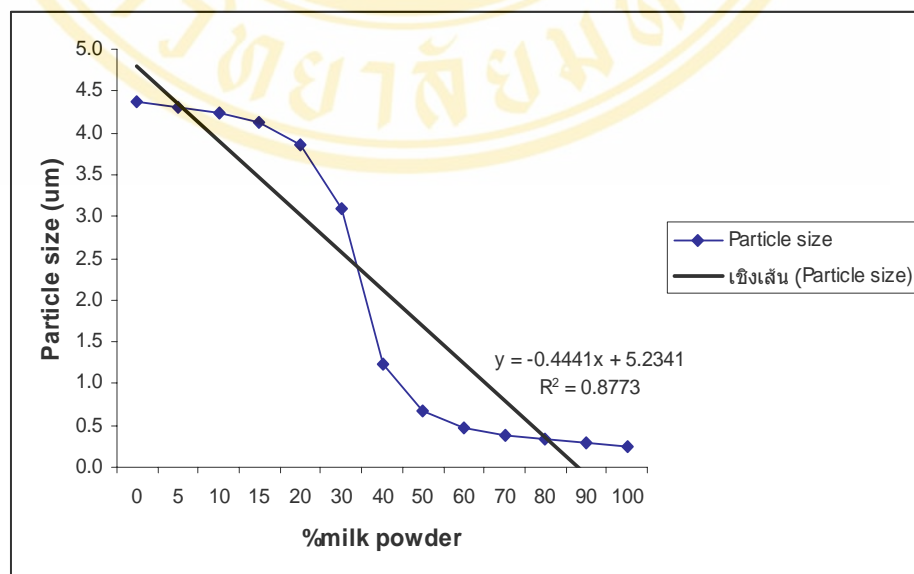


Figure 26 Milk fat particle size in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder.

**4.4.2 Milk fat particle size in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.**

Table 26 Milk fat particle size in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

| %SMP*       | Milk fat particle size (µm) |
|-------------|-----------------------------|
| 100% RM     | 4.372                       |
| RM + 5%SMP  | 4.342                       |
| RM + 10%SMP | 4.397                       |
| RM + 15%SMP | 4.403                       |
| RM + 20%SMP | 4.333                       |
| RM + 30%SMP | 4.214                       |
| RM + 40%SMP | 3.868                       |
| RM + 50%SMP | 3.452                       |
| RM + 60%SMP | 2.675                       |
| RM + 70%SMP | 0.496                       |
| RM + 80%SMP | 0.173                       |
| RM + 90%SMP | 0.138                       |
| 100% SMP    | 0.124                       |

\* %SMP = percentage of reconstituted skimmed milk powder

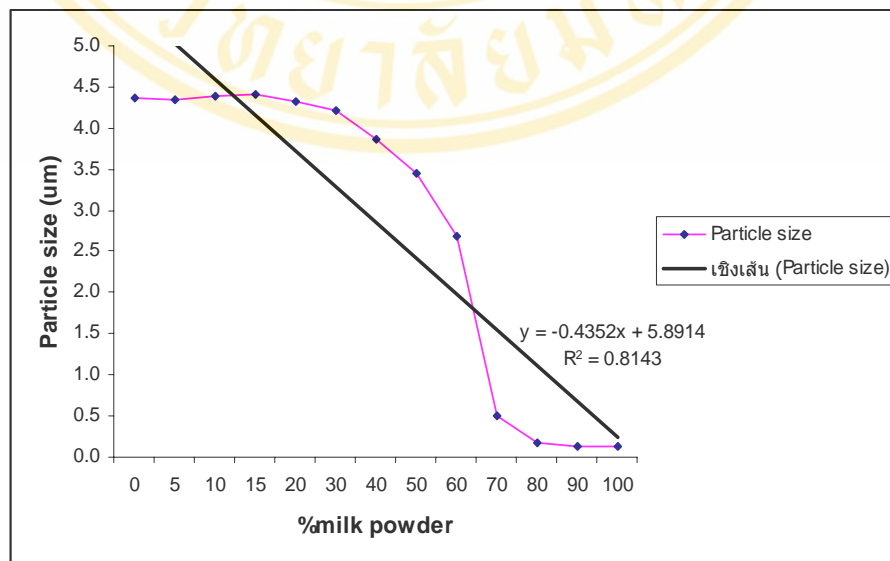


Figure 27 Milk fat particle size in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder.

Table 27 Milk fat particle size in pasteurized milk samples purchased from supermarkets in Bangkok.

| Company             | Milk fat particle size ( $\mu\text{m}$ ) |
|---------------------|--|
| <b>Whole milk</b>   |  |
| A                   | 0.254                                    |
| B                   | 0.278                                    |
| C                   | 0.208                                    |
| D                   | 0.197                                    |
| E                   | 0.286                                    |
| <b>Low-fat milk</b> |  |
| A                   | 0.161                                    |
| B (skimmed milk)    | 0.132                                    |
| C                   | 0.156                                    |
| D                   | 0.165                                    |
| E                   | 0.158                                    |

Table 28 Milk fat particle size in UHT milk samples purchased from supermarkets in Bangkok.

| Company             | Milk fat particle size ( $\mu\text{m}$ ) |
|---------------------|--|
| <b>Whole milk</b>   |  |
| A                   | 0.228                                    |
| B                   | 0.192                                    |
| C                   | 0.188                                    |
| D                   | 0.201                                    |
| E                   | 0.201                                    |
| F                   | 0.164                                    |
| G                   | 0.213                                    |
| <b>Low-fat milk</b> |  |
| A                   | 0.157                                    |
| B                   | 0.142                                    |

## CHAPTER V

### DISCUSSION

#### 5.1 Determination of deoxyketone content in milk samples

Deoxyketone content in milk samples was determined by colorimetric method. The results indicated a significant difference ( $p < 0.05$ ) in deoxyketone content of both groups of combined milk (raw milk combined with reconstituted whole milk powder and raw milk combined with reconstituted skimmed milk powder). The results showed higher absorbance of deoxyketone content in raw milk combined with reconstituted skimmed milk powder than that in raw milk combined with reconstituted whole milk powder. Higher ratios of milk powder to raw milk showed higher contents of deoxyketone than those of lower ratio. It was found that, raw milk combined with reconstituted whole milk powder (0-100%) contained 0.32-2.95 of absorbance of deoxyketone whereas raw milk combined with reconstituted skimmed milk powder (0-100%) contained 0.36-6.18 of absorbance of deoxyketone content (Table 13 and Table 14).

The method was used to determine deoxyketone content in market fresh milk (pasteurized milk and UHT milk) purchased from supermarket. The results could be concluded as follows.

1. Pasteurized milk of company A, B, D, and E

Pasteurized milk samples of company A, B, D, and E were found to have 1.329, 1.273, 1.238, and 1.354 of absorbance of deoxyketone, respectively. Thus, pasteurized milk of company A, B, D, and E may be produced by combining 15-40% of reconstituted milk powder or may be produced at a higher temperature than that used for pasteurization.

2. Pasteurized milk of company C

Pasteurized milk of company C was found to have 1.519 of absorbance of deoxyketone. This indicated that pasteurized milk of company C may be produced by

combining raw milk with approximately 20-50% reconstituted milk powder or may be produced at a higher temperature than that used for pasteurization.

### 3. Pasteurized low-fat milk of company A and C

Pasteurized low-fat milk samples of company A and C were found to have 1.826 and 2.027 of absorbance of deoxyketone, respectively. The result indicated that pasteurized low-fat milk of company A and C may be produced by combining 30-60% reconstituted milk powder or produced at a higher temperature than that used for pasteurization.

### 4. Pasteurized low-fat milk of company B

The absorbance of deoxyketone content of pasteurized low-fat milk of company B was found to have 1.127. Therefore, the sample may be produced from raw milk combined with approximately 15-40% reconstituted milk powder or may be produced at a temperature higher than that used for pasteurization.

### 5. Pasteurized low-fat milk of company D

Pasteurized low-fat milk of company D contained 0.927 of absorbance of deoxyketone. The result indicated that it may be produced by combining approximately 15-30% reconstituted milk powder with fresh milk or may be produced at a temperature higher than that used for pasteurization.

### 6. Pasteurized low-fat milk of company E

The deoxyketone content of pasteurized low-fat milk company E was found to be 0.810 of absorbance of deoxyketone. Thus, the product of company E may be produced by combining approximately 5-20% reconstituted milk powder with fresh milk or may be produced at a temperature higher than that used for pasteurization.

The absorbance of deoxyketone contents in UHT milk products of company A, B, C, D, E, F, and G were in range of 1.902–3.800. These indicated that the UHT milk products may be produced by combining approximately 40-100% of reconstituted milk powder.

There were a few reports on the deoxyketone content in milk samples. The minimum concentration of added milk powder in raw milk detectable by deoxyketone determination was 0.89% (Wanarat, 2001). Heat treatment results in many chemical and structural changes in milk, the extent of which depends on the temperature and duration of heating (Evangelisli et al, 1999; Villamiel and Corzo, 1998). One mechanism involved in the deleterious consequence of heat treatment is the maillard reaction that leads to the decrease in nutritional value of proteins and formation of brown compounds in milk (Asylbek and Eric, 2002). The first stable product of this reaction is deoxyketone or lactulosyllysine, which leads to a loss of nutritional value due to blockage of the essential amino acid lysine (Aragon et al, 2004). The effect of the maillard reaction depends on the following factors.

1. pH and Buffers. The maillard reaction can develop in acidic or alkaline media, although it is favored under alkaline conditions, where the amine groups of the amino acids, peptides, and proteins are in the basic form. Increasing the pH also ensures that more of the hexoses are in the open chain or reducing form (Burton and McWeeney, 1963). Several studies have reported an increase in reaction rate as the pH increases. Thus, foods of high acidity are less susceptible to these reactions. Ionic strength of buffers has been shown to increase the rate of browning for sugar-amino acid systems as a result the influence of the ionic environment in which the reaction takes place (Eskin, 1990).

2. Temperature. The temperature dependence of this reaction has been demonstrated in a number of quantitative studies, where increased rates were reported with rise in temperature (Eskin, 1990).

3. Moisture content. The maillard reaction proceeds rapidly in a solution, although complete dehydration or excessive moisture levels inhibit this process (Wolfrom and Rooney, 1953). An increase in humidity was also shown by Dworschak and Hegedus (1974) to cause an increase in the loss of lysine and tryptophan with the concomitant increase in hydroxymethylfurfural (HMF) formation and browning. In general, it appears that this reaction is favored at an optimum moisture content corresponding to fairly low moisture levels (Danehy, 1986).

4. Sugars. Reducing sugars are essential ingredients in this reaction, providing the carbonyl groups for the interaction with the free amino groups of amino

acids, peptides, and proteins. The initial rate of this reaction is dependent on the rate at which the sugar ring opens to the oxo or reducible form (Eskin, 1990).

5. Metals. The formation of metal complexes with amino acids can influence the maillard reaction. This reaction was catalyzed by copper and iron, while manganese and in inhibit this reaction (Ellis, 1959; Markuze, 1963).

In this experiment, deoxyketone analysis was developed to determine deoxyketone content as an indicator of the addition of reconstituted whole milk powder or skimmed milk powder to raw milk and market fresh milk (pasteurized milk and UHT milk). The results indicated that deoxyketone content could be employed to detect the addition of reconstituted whole milk powder and reconstituted skimmed milk powder in raw milk, pasteurized milk and UHT milk. However, since deoxyketone is one of the maillard products, the temperature during processing many have impact on deoxyketone content in pasteurized and UHT milk. It may be effect to higher deoxyketone content than the reality.

## **5.2 Biochemical analysis in milk samples**

The contents of milk composition in raw milk combined with reconstituted whole milk powder and skimmed milk powder are measured by using Milkocsan™ 4000. The quantity of milk composition in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder and skimmed milk powder indicated resemble results. In samples prepared from raw milk combined with reconstituted whole milk powder almost of factors increased to conform to the higher percentage of milk powder except fat content which decreased. This could also observe in samples prepared from raw milk combined with reconstituted skimmed milk powder. Nevertheless in raw milk combined with reconstituted skimmed milk powder was showed more obviously trend in each factors than raw milk combined with reconstituted whole milk powder.

The results of biochemical analysis might not be clearly discriminating the differences between raw milk and combined milk. Therefore, to overcome this problem the ratios of milk composition were set up in this study.

### **5.3 Determination of the ratios of milk composition in milk samples**

#### **5.3.1 Ratios of milk composition in samples prepared by combining raw milk with various amounts of reconstituted whole milk powder**

From the biochemical analysis 42 ratios of milk composition were calculated. Among these, six ratios were chosen to differentiate between fresh milk and fresh milk combined with reconstituted whole milk powder. The distributions of six ratios were shown in Table 21 and Table 22 including Fat/Urea, Fat/Cit.Ac, Protein/Urea, Lactose/Urea, SNF/Urea, and TS/Urea. Almost of ratios were selected contain urea for the significant of urea in milk is used as an indicator for protein degradation, which reflects the shelf life of milk. The linear regression and regression coefficient ( $R^2$ ) represent in Figure 14 to Figure 25.

From this result, all of six ratios indicated high regression coefficient ( $>0.95$ ), while Fat/Urea was the highest one (0.9738). For linear regression, ratios of TS/Urea were shown to be the highest value. Statistical analysis showed that the Fat/Cit.Ac ratios of different amount reconstituted whole milk powder added were significantly different ( $p < 0.05$ ).

#### **5.3.2 Ratios of milk composition in samples prepared by combining raw milk with various amounts of reconstituted skimmed milk powder**

There were six ratios of milk composition (Table 22) selected to differentiate between fresh milk and fresh milk combined with reconstituted skimmed milk powder. All of ratios were decreased consecutively while percentage of skimmed milk powder was higher. The linear regression and regression coefficient ( $R^2$ ) were shown in Figure 15, 17, 19, 21, 23, and 25. The results, showed that Fat/Cit.Ac provided the best value of  $R^2$  (0.9955), whereas TS/Urea provided the best value of linear regression equation ( $y = -35.737x + 470.38$ ) with the highest slope when compared to other ratios. Statistical analysis showed that all of six ratios selected were significantly different among different amount of skimmed milk powder added.

In this experiment, ratios of milk compositions were developed to determine the difference between fresh milk and fresh milk combined with reconstituted milk powder (whole milk powder and skimmed milk powder), as an indicator of the addition of whole milk powder or skimmed milk powder to pasteurize and UHT milk.

From this study, the ratios of milk composition exhibited more differentiating power than biochemical analysis. Therefore this method could be used to identify milk samples which may be combined with reconstituted milk powder.

At present, skimmed milk powder might be added into raw milk during processing rather than whole milk powder in order to maintain fat content of the products. In addition the cost of skimmed milk powder, as a raw material, was cheaper than whole milk powder. For the most suitable ratio that can be apply or use as an index for detect fresh milk and fresh milk combined with reconstituted milk powder depend on several factors including period of time for measure, complicated or easy method, cost, instrument support, safety, etc.

However, fresh milk is divided into three groups i.e. whole milk, low-fat milk or partially skimmed milk, and skimmed milk based on percentage of milk fat and solid-not-fat (SNF). Whole milk must contain at least 3.2% by weight of milk fat and at least 8.25% by weight of solid-not-fat. Low-fat milk contains 0.1% to 3.2% by weight of milk fat and not less than 8.5% by weight of solid-not-fat. Skimmed milk contains not less than 0.1% by weight of milk fat and at least 8.8% by weight of solid-not-fat. All of them must contain not less than 2.8% by weight of milk protein. Therefore, the parameters used to calculate the ratios that can be applied as an indication of addition of reconstituted milk into pasteurized milk and UHT milk should exclude fat, protein, and solid-not-fat due to bylaw of Notification of the Ministry of Public Health No.265 (B.E. 2545) as described above. In this study, there were six ratios exhibited difference values between fresh milk and fresh milk combined with milk powder. Nevertheless, there were only two ratios i.e. Lactose/Urea and TS/Urea that could be apply for detect addition of reconstituted milk in pasteurized milk and UHT milk. Besides, all of six ratios could be apply to investigate the addition of reconstituted milk powder into unprocessed milk or raw milk.

### **5.3.3 Ratios of milk composition in pasteurized milk purchased from supermarket in Bangkok**

In this study, ratios of Lactose/Urea and TS/Urea denoted similar  $R^2$  but TS/Urea showed the best linear regression. Therefore TS/Urea was used to differentiate between fresh milk and fresh milk combined with reconstituted milk

powder. The TS/Urea values in pasteurized milk samples purchased from supermarket could be concluded as follows.

1. Pasteurized whole milk of companies A and B

TS/Urea of whole fresh milk of companies A and B were found to be 373.46 and 374.30. When compared these values to those in Table 22 whole fresh milk of companies A and B might be produced by combining 5-10% of reconstituted milk powder with raw milk during production.

2. Pasteurized whole milk companies C, D, and E

TS/Urea of whole fresh milk of companies C, D, and E were found to be 312.21, 306.63, and 339.22, respectively. When compared these values to those in Table 22 whole fresh milk of companies C, D, and E might be produced by combining 10-15% of reconstituted milk powder with raw milk during production.

3. Pasteurized low-fat milk of companies A and E

TS/Urea of low-fat milk of companies A and E were found to be 160.52 and 140.41, respectively. When compared these values to those in Table 22 low-fat milk of companies A and E might be produced by combining 40-50% of reconstituted milk powder with raw milk during production.

4. Pasteurized low-fat milk of company B

TS/Urea of low fat milk of company B was found to be 121.30. When compared these values to those in Table 22 low-fat milk of company B might be produced by combining 60-70% of reconstituted milk powder with raw milk during production.

5. Pasteurized low-fat milk of company C

TS/Urea of low-fat milk of company C was found to be 122.71. When compared these values to those in Table 22 low-fat milk of company C might be produced by combining 50-60% of reconstituted milk powder with raw milk during production.

6. Pasteurized low-fat milk of company D

TS/Urea of low-fat milk of company D was found to be 187.55. When compared these values to those in Table 22 low-fat milk of company D might be produced by combining 30-40% of reconstituted milk powder with raw milk during production.

### 5.3.4 Ratios of milk composition in UHT milk samples purchased from supermarket in Bangkok

Based on the results of TS/Urea in UHT milk that purchased from supermarket can be concluded as follow:

1. UHT whole milk of companies A and E

TS/Urea of whole milk of company A and E were found to be 226.88 and 246.68. When compared these values to those in Table 22 whole milk of companies A and E might be produced by combining 20-30% of reconstituted milk powder with raw milk during production.

2. UHT whole milk of companies B, D and F

TS/Urea of whole milk of companies B, D and F were found to be 329.18, 322.51 and 305.21, respectively. When compared these values to those in Table 22 whole milk of companies B, D, and F might be produced by combining 10-15% reconstituted milk powder with raw milk during production.

3. UHT whole milk of company C

TS/Urea of whole milk of company C was found to be 361.51. When compared these values to those in Table 22 whole milk of company C might be produced by combining 5-10% of reconstituted milk powder with raw milk during production.

4. UHT whole milk of company G

TS/Urea of low-fat milk of company G was found to be 280.25. When compared these values to those in Table 22 whole milk of company G might be produced by combining 15-20% of reconstituted milk powder with raw milk during production.

5. UHT low-fat milk of company A

TS/Urea of low-fat milk of company A was found to be 112.10. When compared these values to those in Table 22 whole milk of company A might be produced by combining 60-70% of reconstituted milk powder with raw milk during production.

## 6. UHT low-fat milk of company B

TS/Urea of low-fat milk of company B was found to be 123.49. When compared these values to those in Table 22 whole milk of company B might be produced by combining 50-60% of reconstituted milk powder with raw milk during production.

### 5.4 Determination of milk fat particle size in milk samples

The results of milk fat particle size in raw milk combining with reconstituted whole milk powder and skimmed milk powder were shown in Table 25 and 26. The results demonstrated decreased in milk fat particle size when the percentage of milk powder was increased in both raw milk combining with various amounts of reconstituted whole milk powder and skimmed milk powder. For milk fat particle size in market fresh milk (pasteurized and UHT milk) were shown in Table 27 and 28. Milk fat particle size in market pasteurized and UHT milk samples showed nearly about 0.1-0.3 $\mu\text{m}$ . that very small when compared with raw milk combining with reconstituted milk powder.

Due to raw milk combining with various amounts of reconstituted whole milk powder and skimmed milk powder not pass homogenize process before milk fat particle size determination that effect to larger size than homogenized milk. In market fresh milk involved homogenization during processing so, it made smaller milk fat particle size than raw milk. Therefore, in this study milk fat particle size could not be used as an index to differentiate the addition of reconstituted whole milk powder or reconstituted skimmed milk powder in market fresh milk (pasteurized and UHT). Nevertheless, milk fat particle size could be used as an index to differentiate between raw milk and raw milk combining with reconstituted milk powder that usefulness for milk factory and cooperation.

## CHAPTER VI

### CONCLUSION

The purpose of this study was to develop an analytical method to determine deoxyketone, biochemical analysis, ratios of milk composition, and milk fat particle size in order to differentiate pure fresh milk from fresh milk combined with reconstituted whole milk powder or skimmed milk powder. The selected parameters were then employed for analyses of market fresh milk products (pasteurized and UHT milk). The results of this study can be concluded as follows.

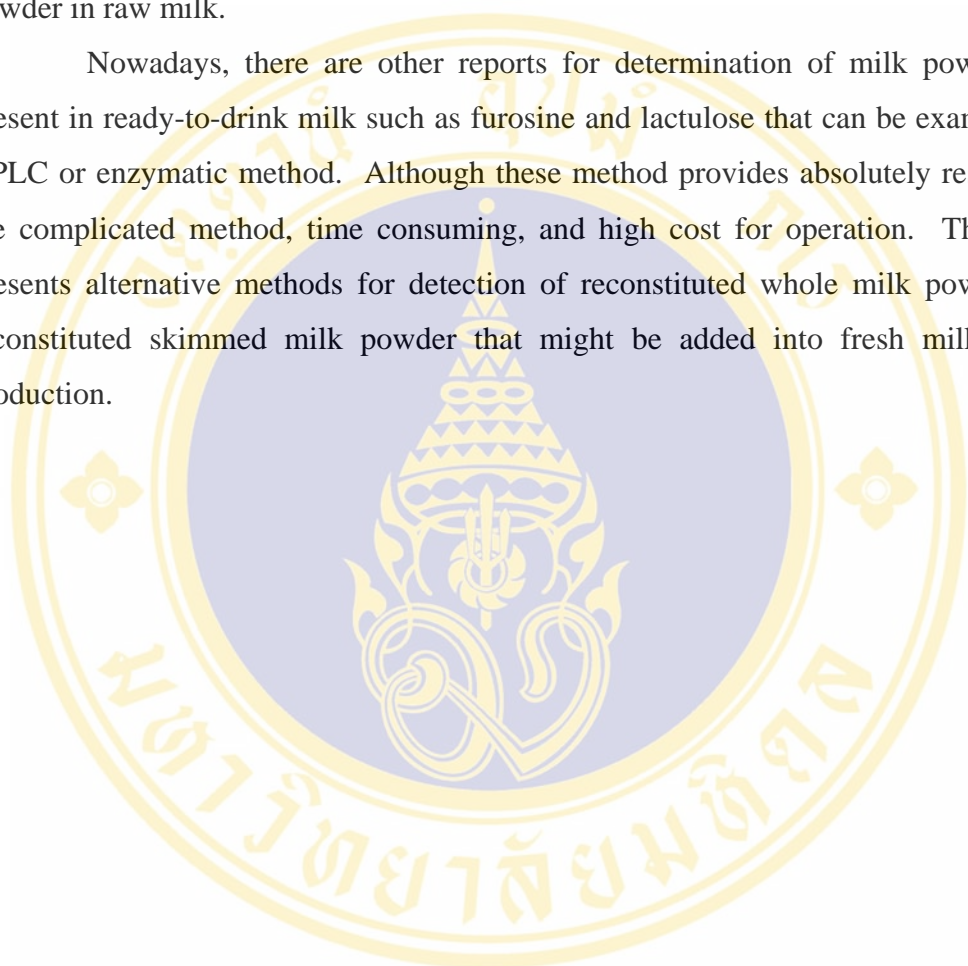
1. Deoxyketone content of raw milk combined with various percentage of reconstituted whole milk powder and skimmed milk powder were significantly different ( $p < 0.05$ ). Nevertheless, it might be unsuitable to be used to discriminate fresh milk from fresh milk combined with reconstituted milk powder in market fresh milk. This is due to the fact that deoxyketone is the product of maillard reaction, heat processing effects higher amount of deoxyketone content. Therefore, deoxyketone in market fresh milk (pasteurized and UHT milk) might be higher than the reality when compared with raw milk combined with reconstituted whole milk powder and reconstituted skimmed milk powder.

2. Biochemical analysis by Milkoscan™ 4000 might not be used to detect the addition of reconstituted whole milk powder or skimmed milk powder in milk samples. Because the content of each factor were found to have similar so, this method could not be able to differentiate fresh milk and fresh milk combined with reconstituted milk powder.

3. Ratios of milk composition could be used as an index for discriminate the addition of reconstituted milk powder (whole milk powder and skimmed milk powder) into raw milk and market fresh milk. In particularly lactose/urea and TS/urea ratios are able to differentiate fresh milk from fresh milk combined with reconstituted milk powder.

4. Milk fat particle size could not be used to detect the addition of reconstituted whole milk powder or skimmed milk powder in market fresh milk. Nevertheless it can be used to differentiate between fresh milk and fresh milk combined with reconstituted whole milk powder and reconstituted skimmed milk powder in raw milk.

Nowadays, there are other reports for determination of milk powder that present in ready-to-drink milk such as furosine and lactulose that can be examined by HPLC or enzymatic method. Although these method provides absolutely result, it is the complicated method, time consuming, and high cost for operation. This study presents alternative methods for detection of reconstituted whole milk powder and reconstituted skimmed milk powder that might be added into fresh milk during production.



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## APPENDIX A

### Particle size in milk samples

#### 1. Particle size in raw milk combined with reconstituted whole milk powder

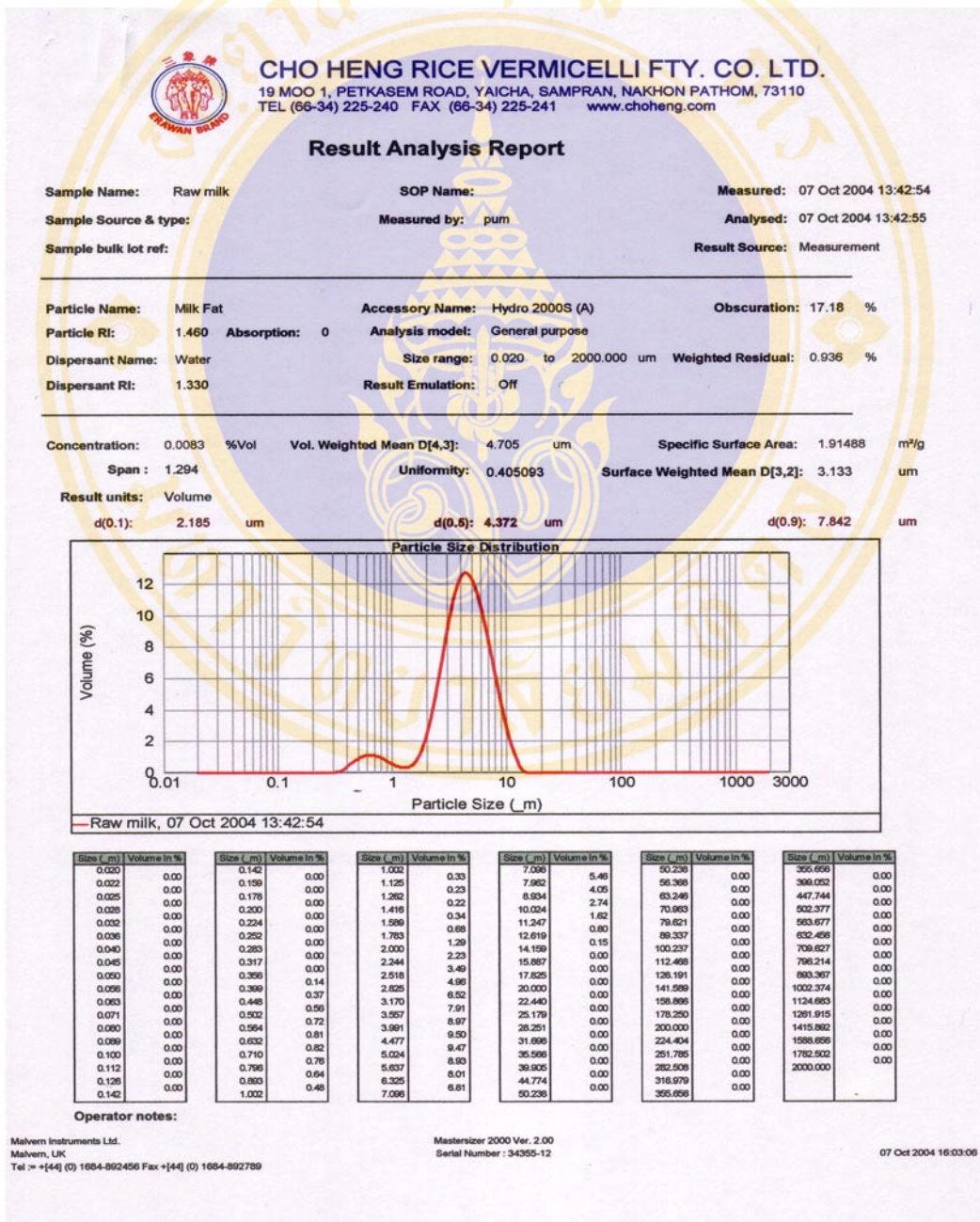


Figure 28 Particle size in raw milk

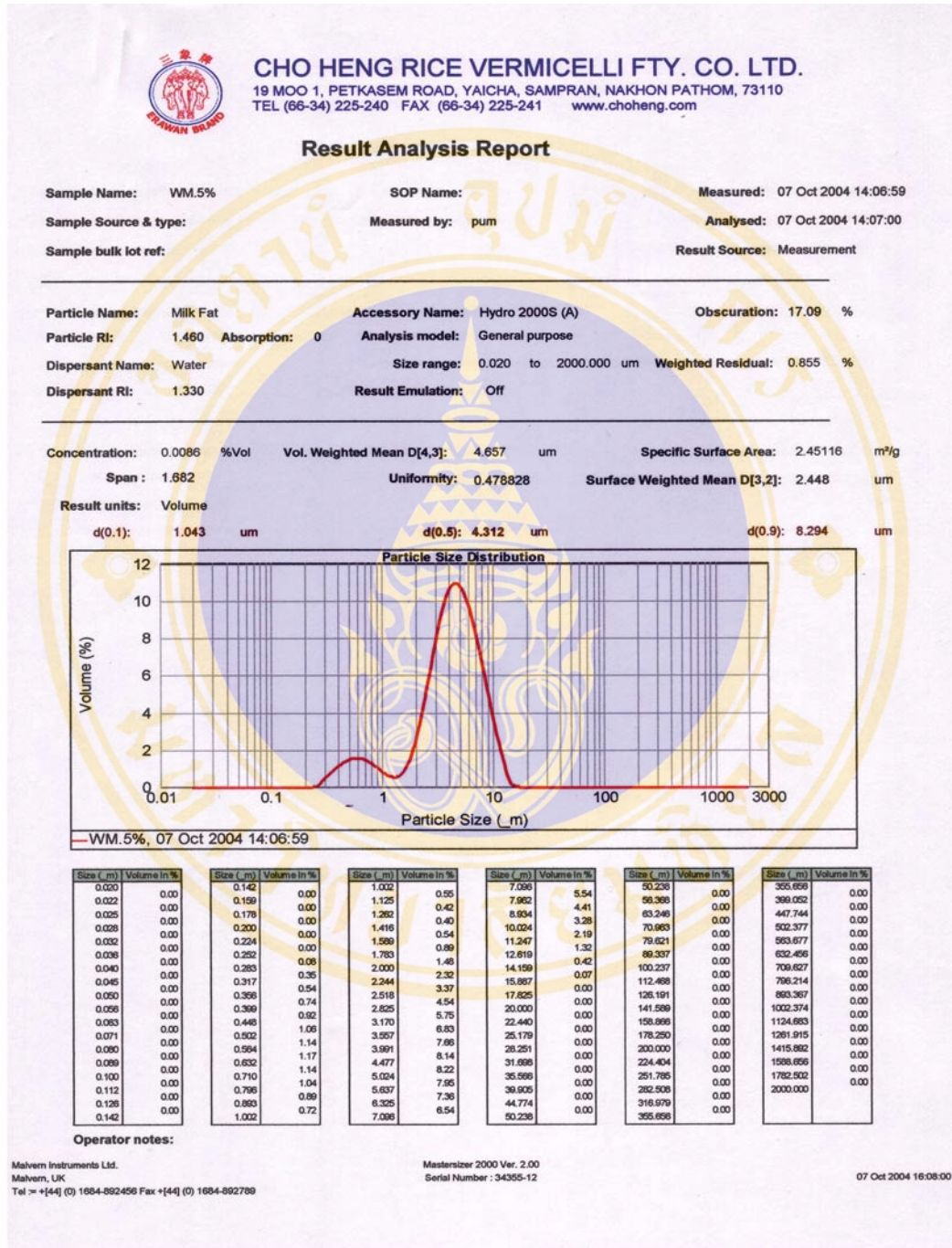


Figure 29 Particle size in raw milk combined with 5% reconstituted WMP

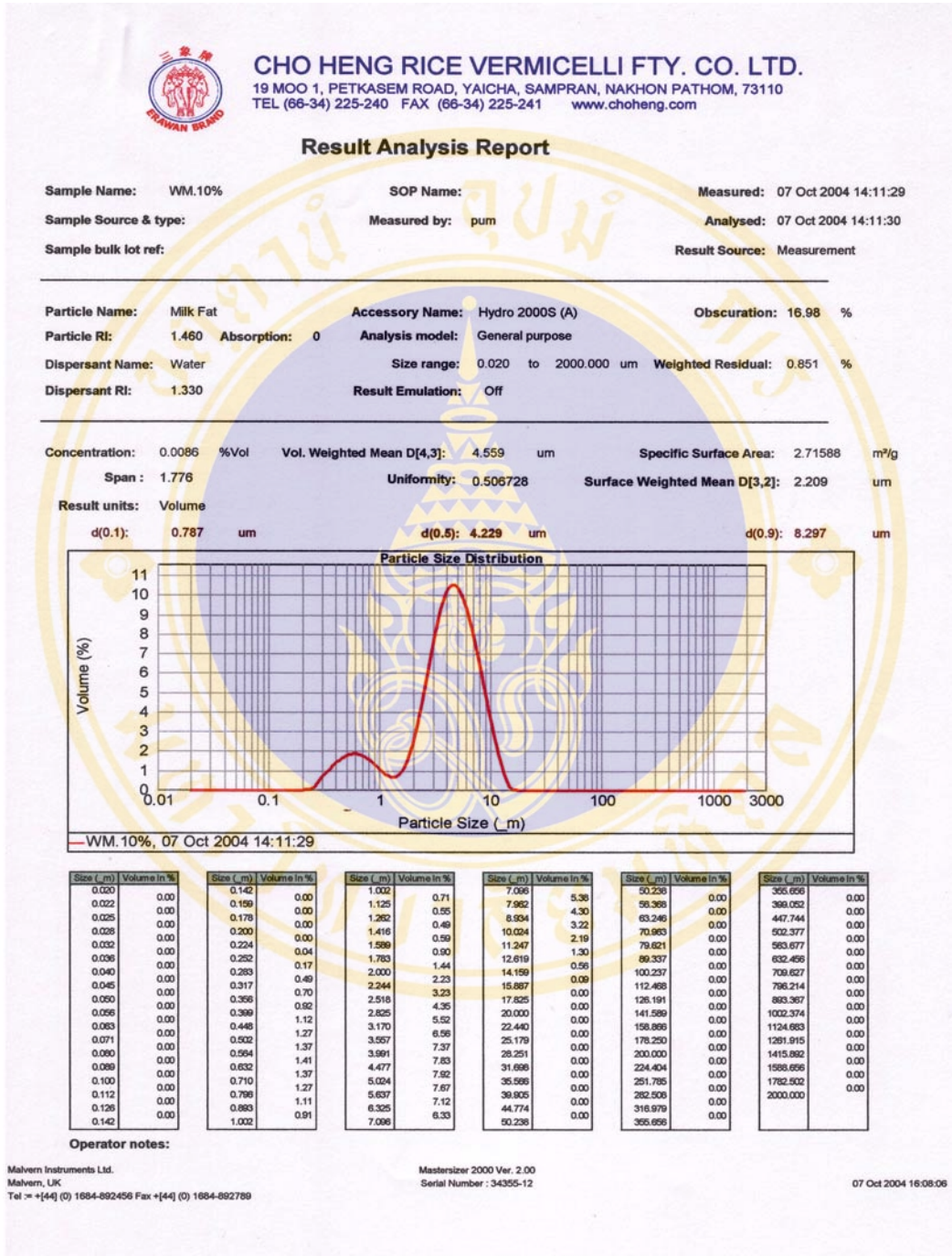


Figure 30 Particle size in raw milk combined with 10% reconstituted WMP

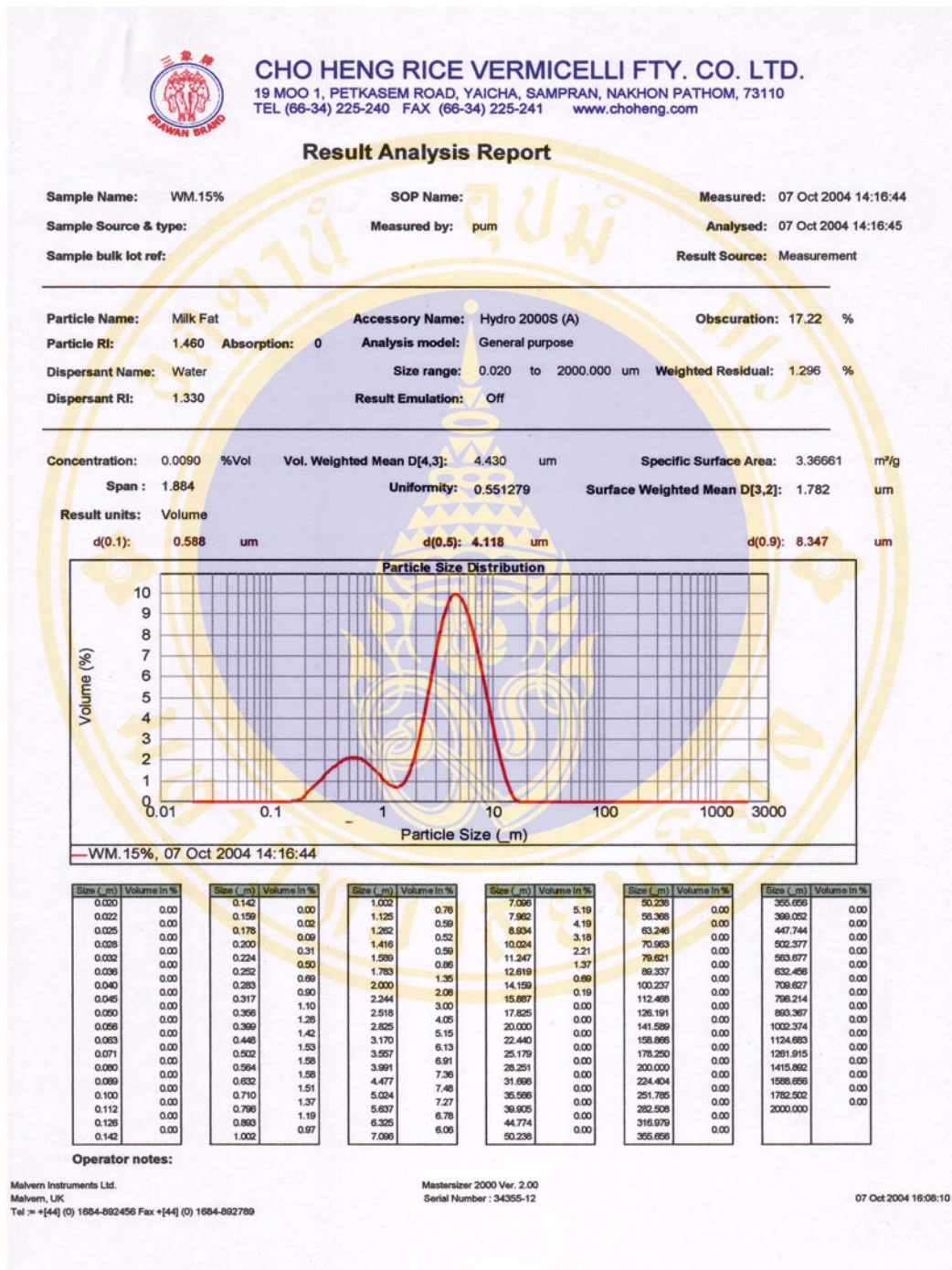


Figure 31 Particle size in raw milk combined with 15% reconstituted WMP

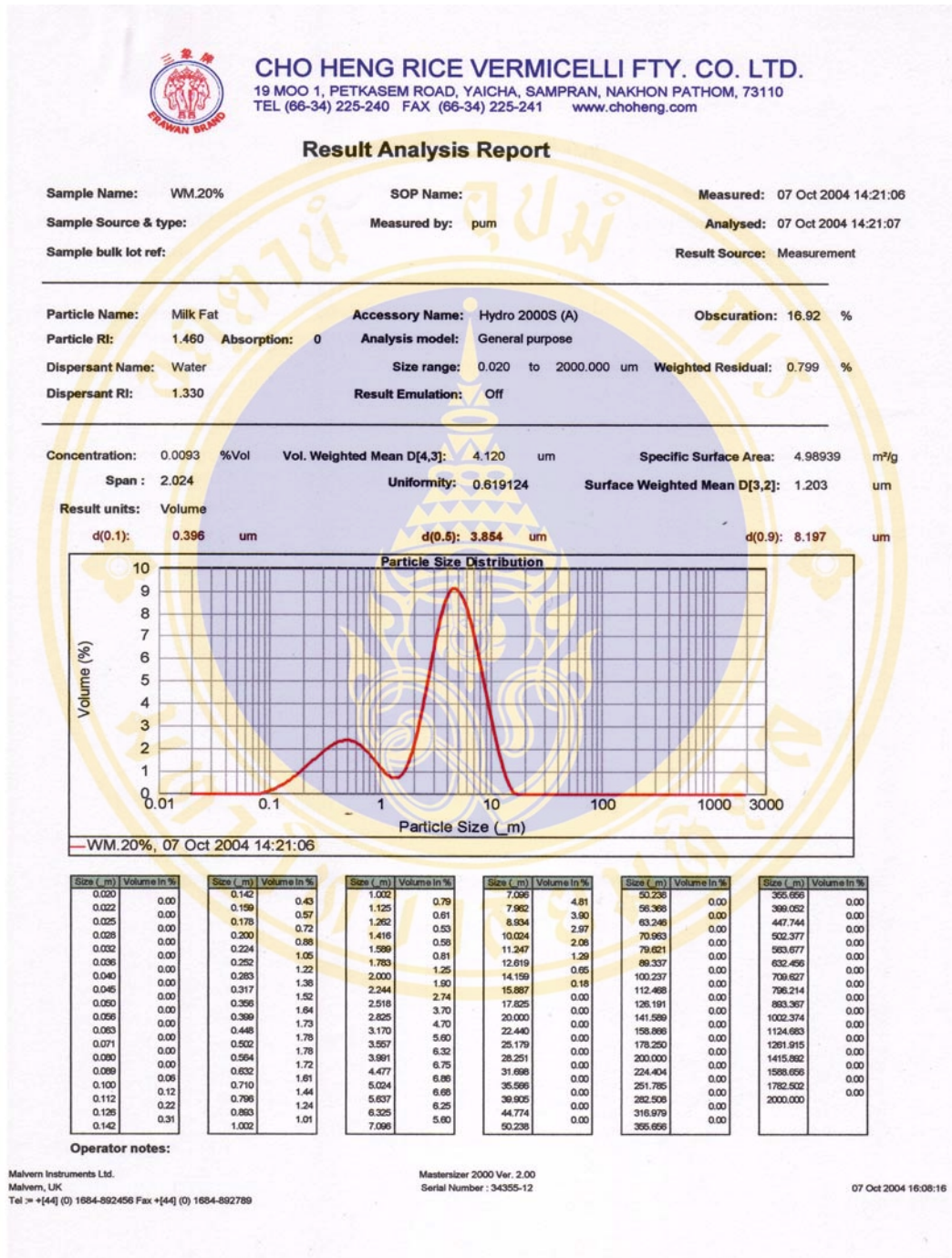


Figure 32 Particle size in raw milk combined with 20% reconstituted WMP

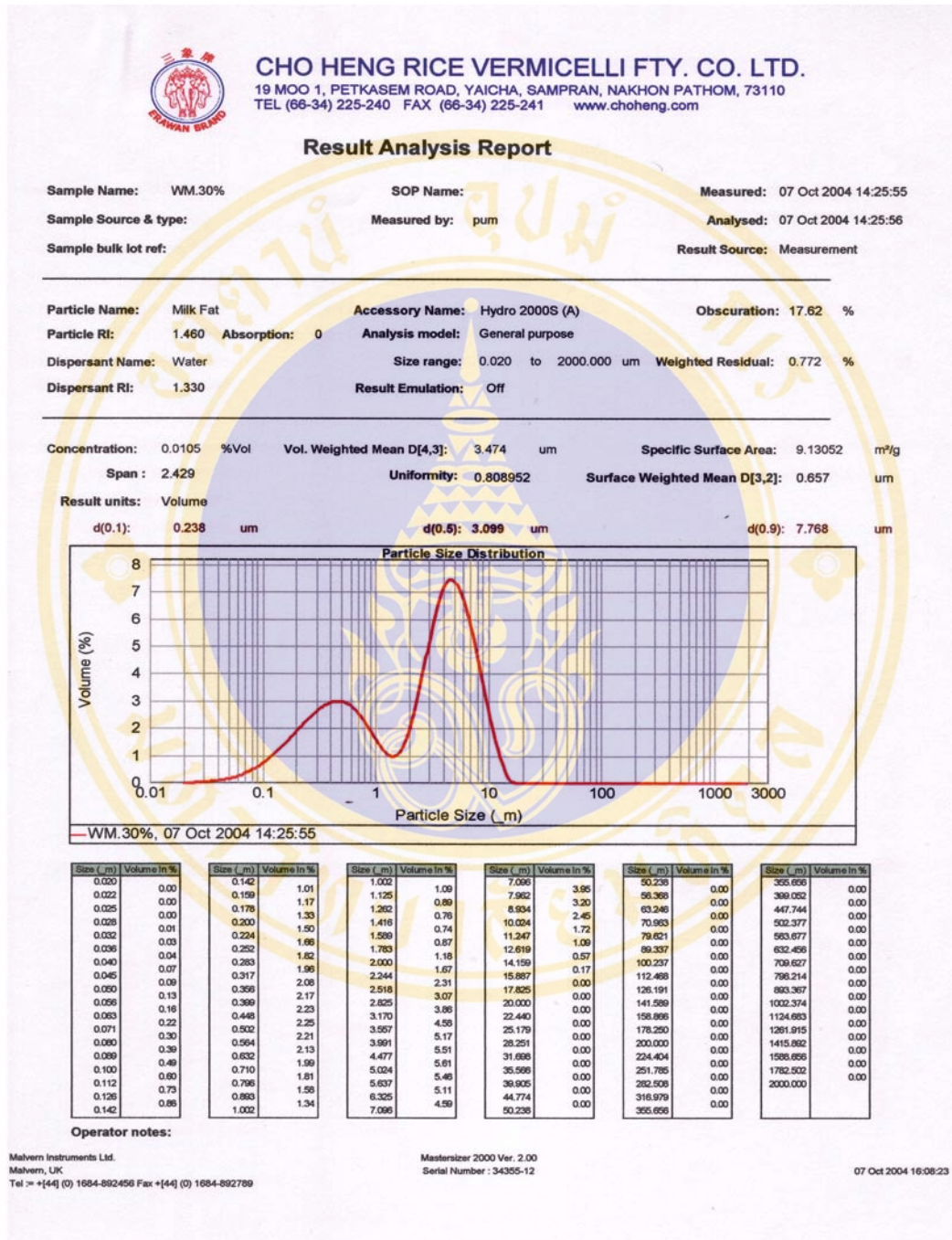


Figure 33 Particle size in raw milk combined with 30% reconstituted WMP

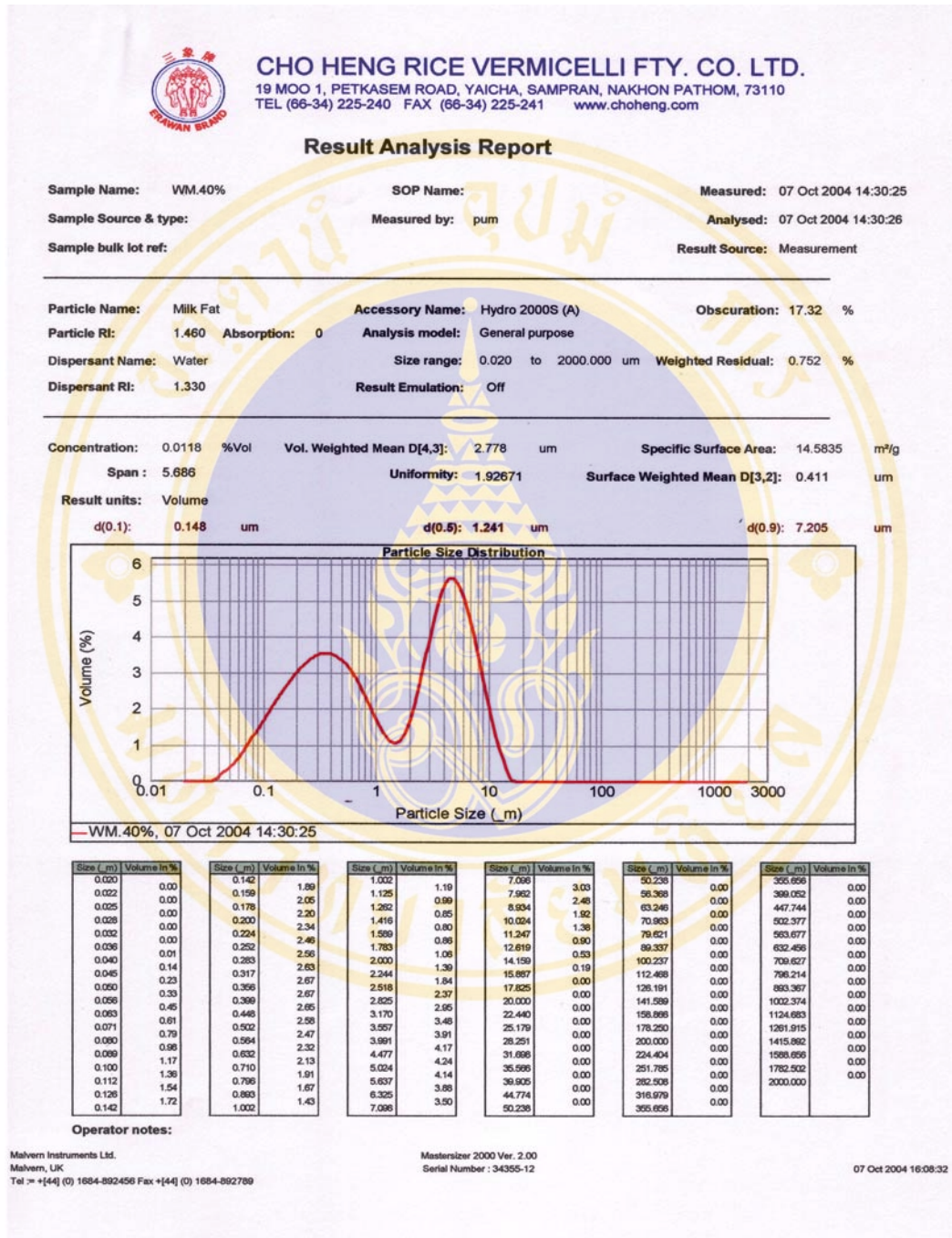


Figure 34 Particle size in raw milk combined with 40% reconstituted WMP

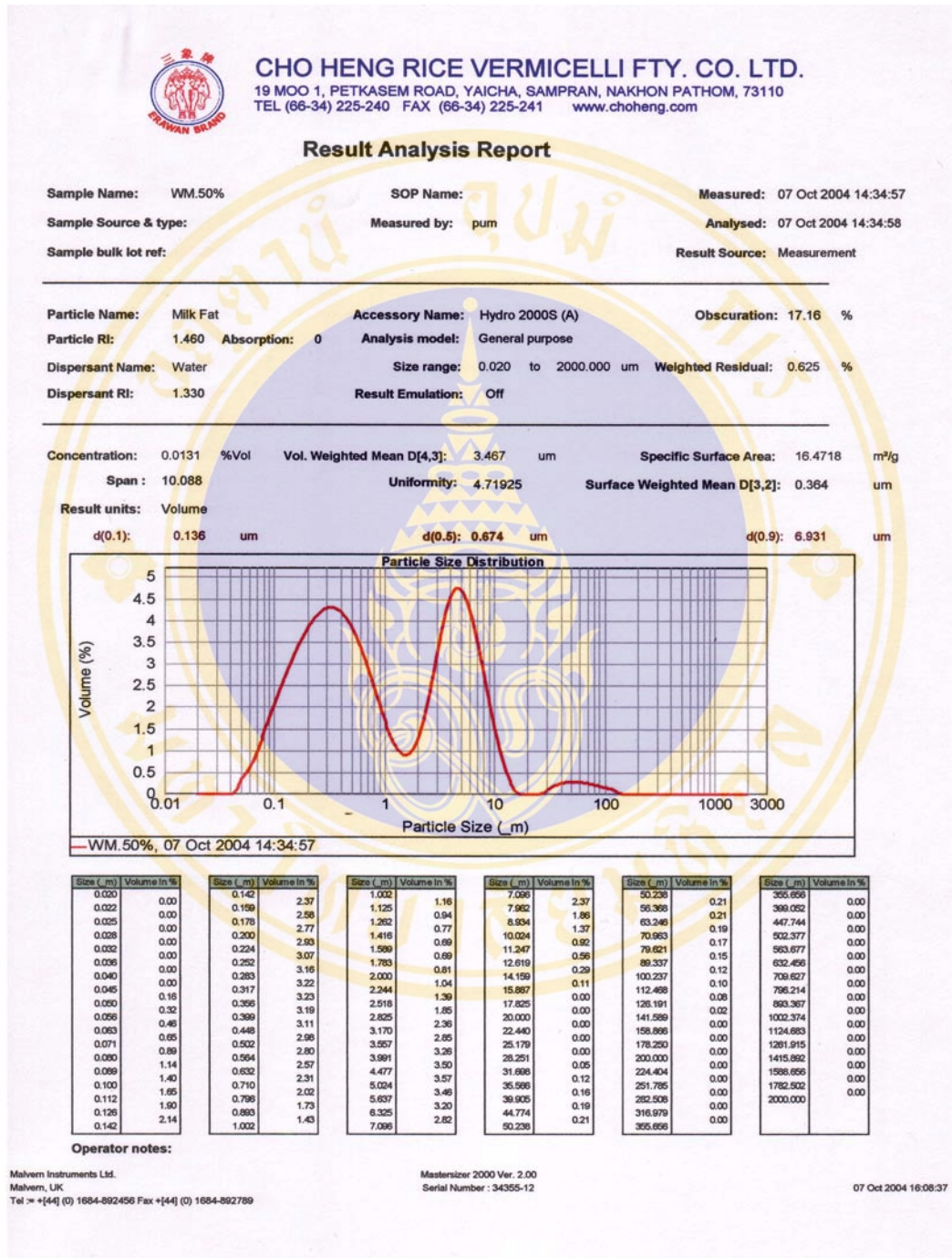


Figure 35 Particle size in raw milk combined with 50% reconstituted WMP

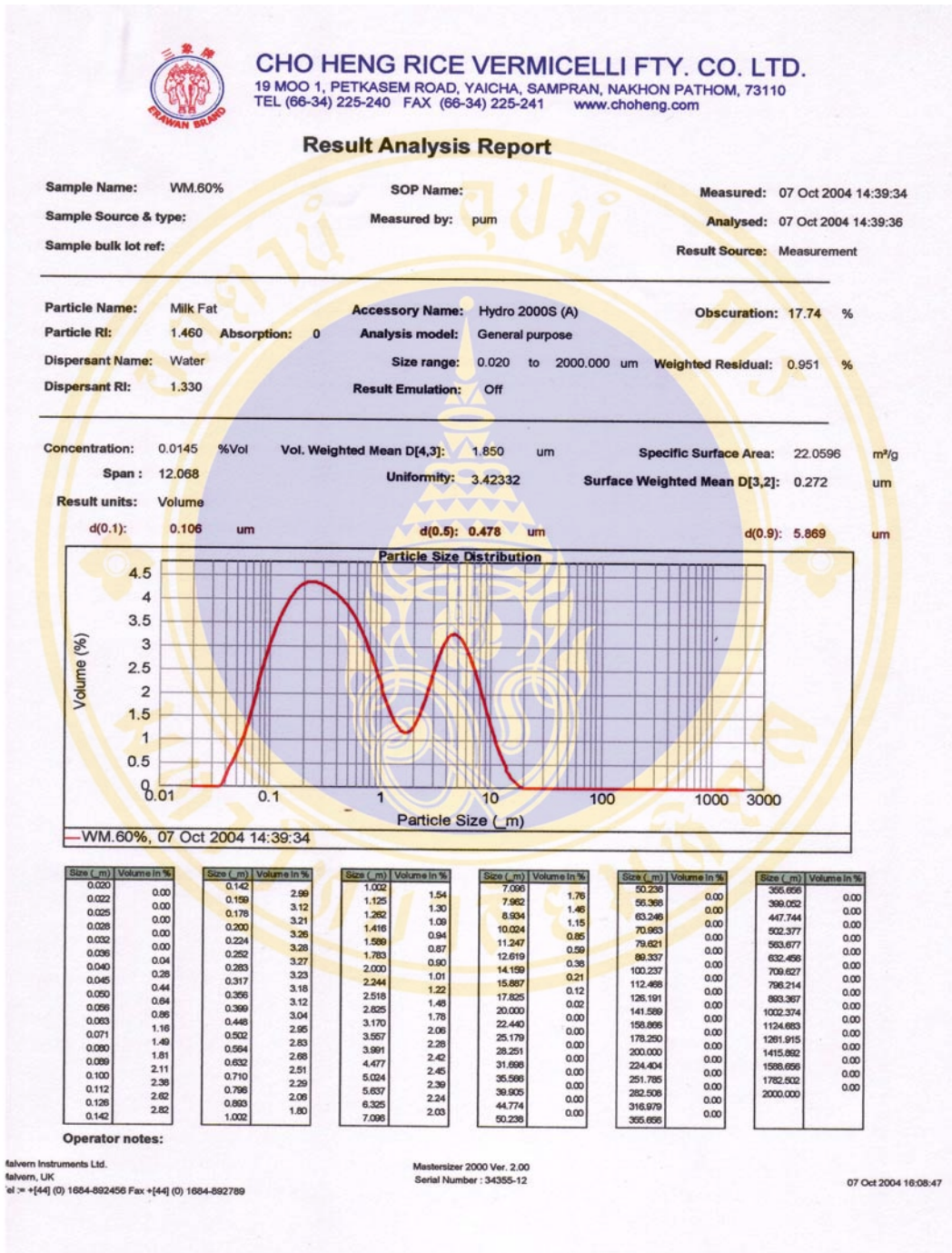


Figure 36 Particle size in raw milk combined with 60% reconstituted WMP

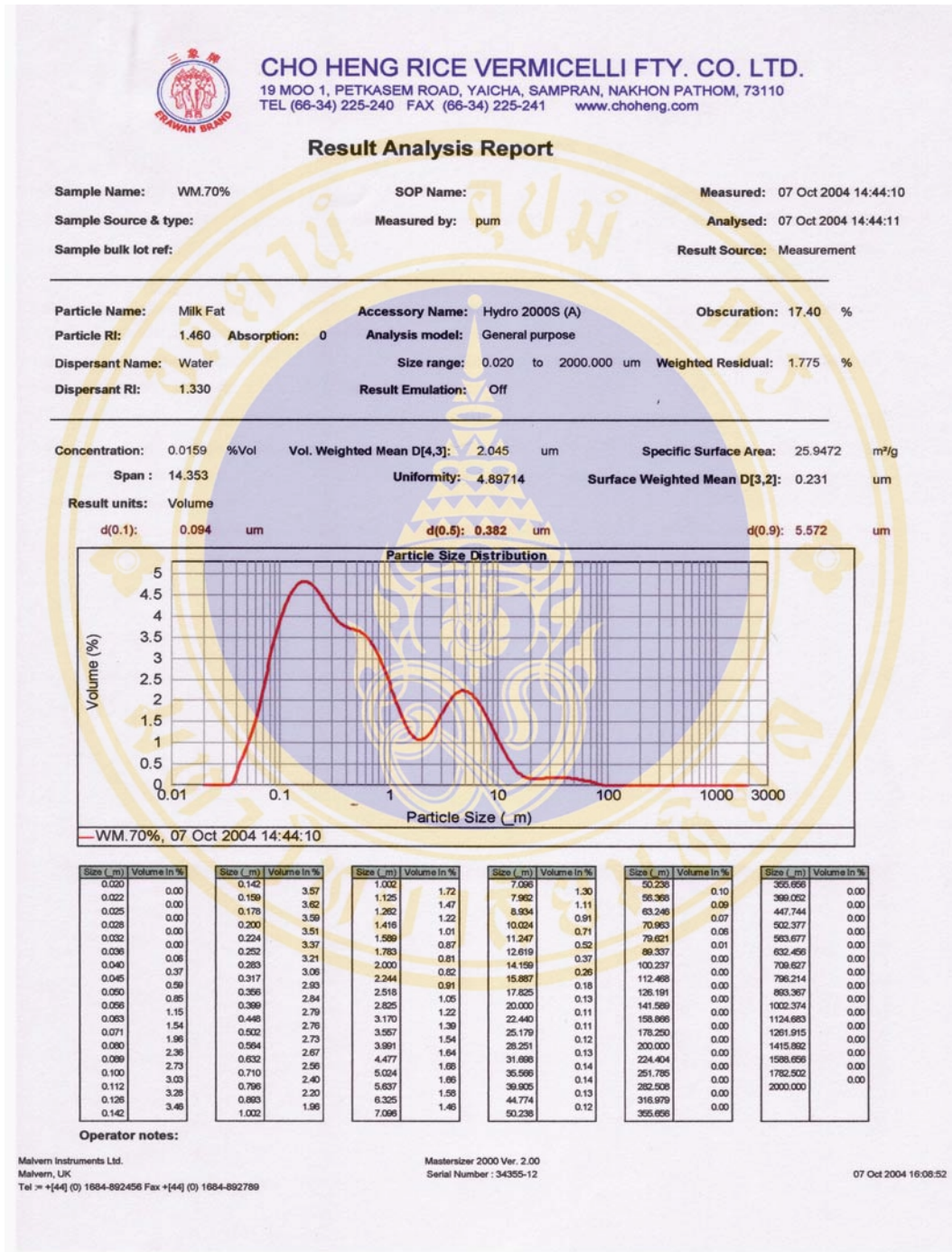


Figure 37 Particle size in raw milk combined with 70% reconstituted WMP

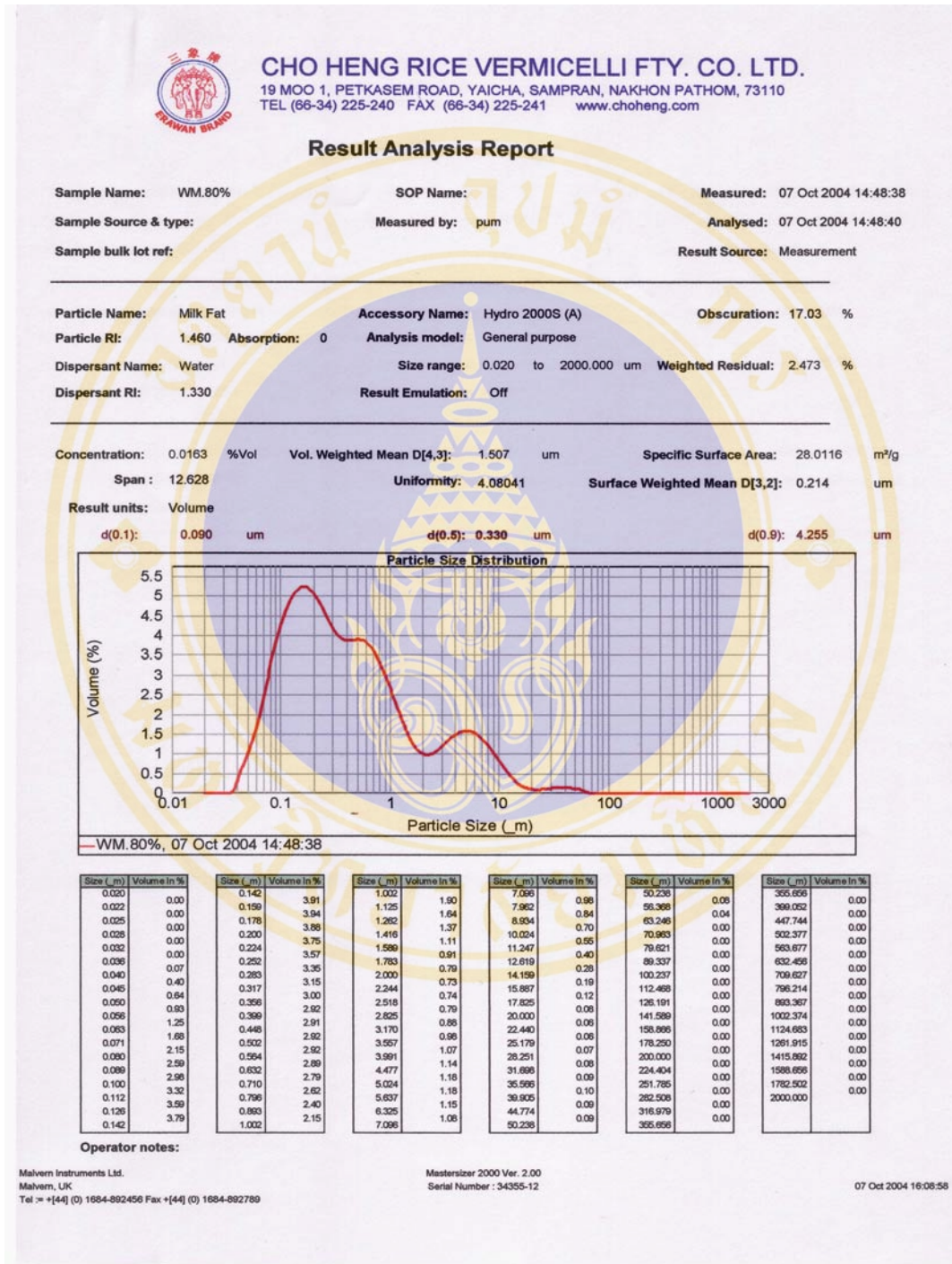


Figure 38 Particle size in raw milk combined with 80% reconstituted WMP

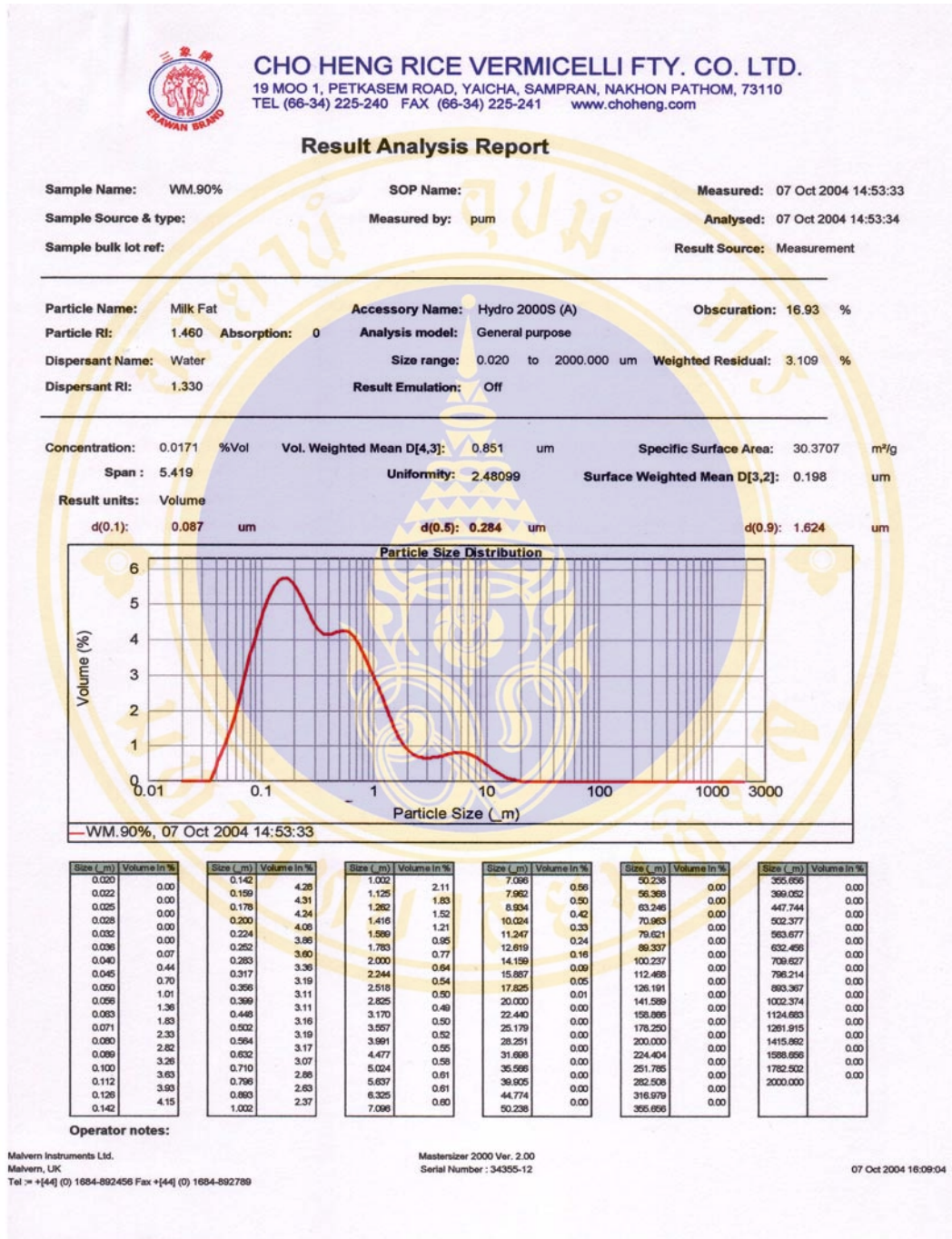


Figure 39 Particle size in raw milk combined with 90% reconstituted WMP

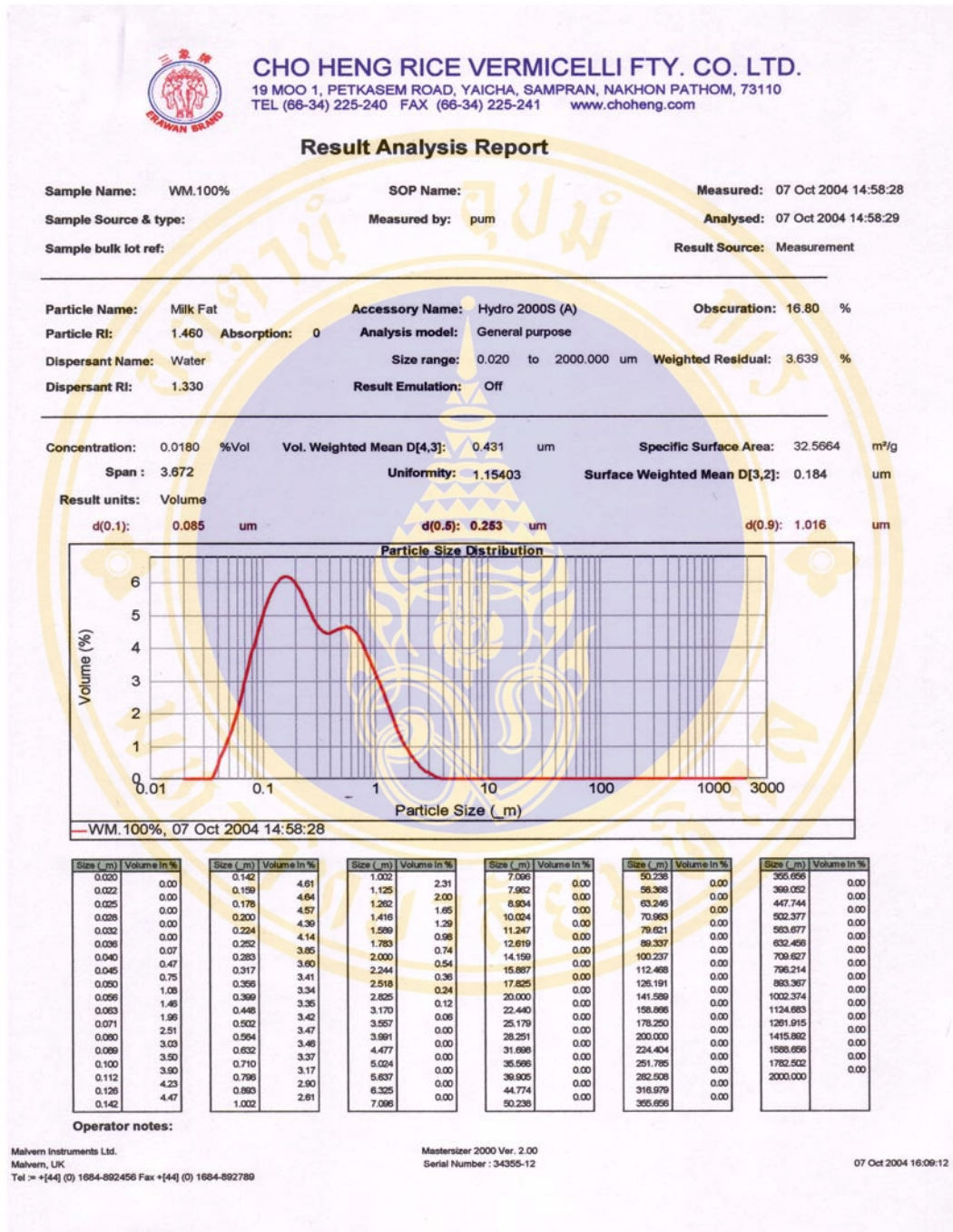


Figure 40 Particle size in raw milk combined with 100% reconstituted WMP

## 2. Particle size in raw milk combined with reconstituted skimmed milk powder

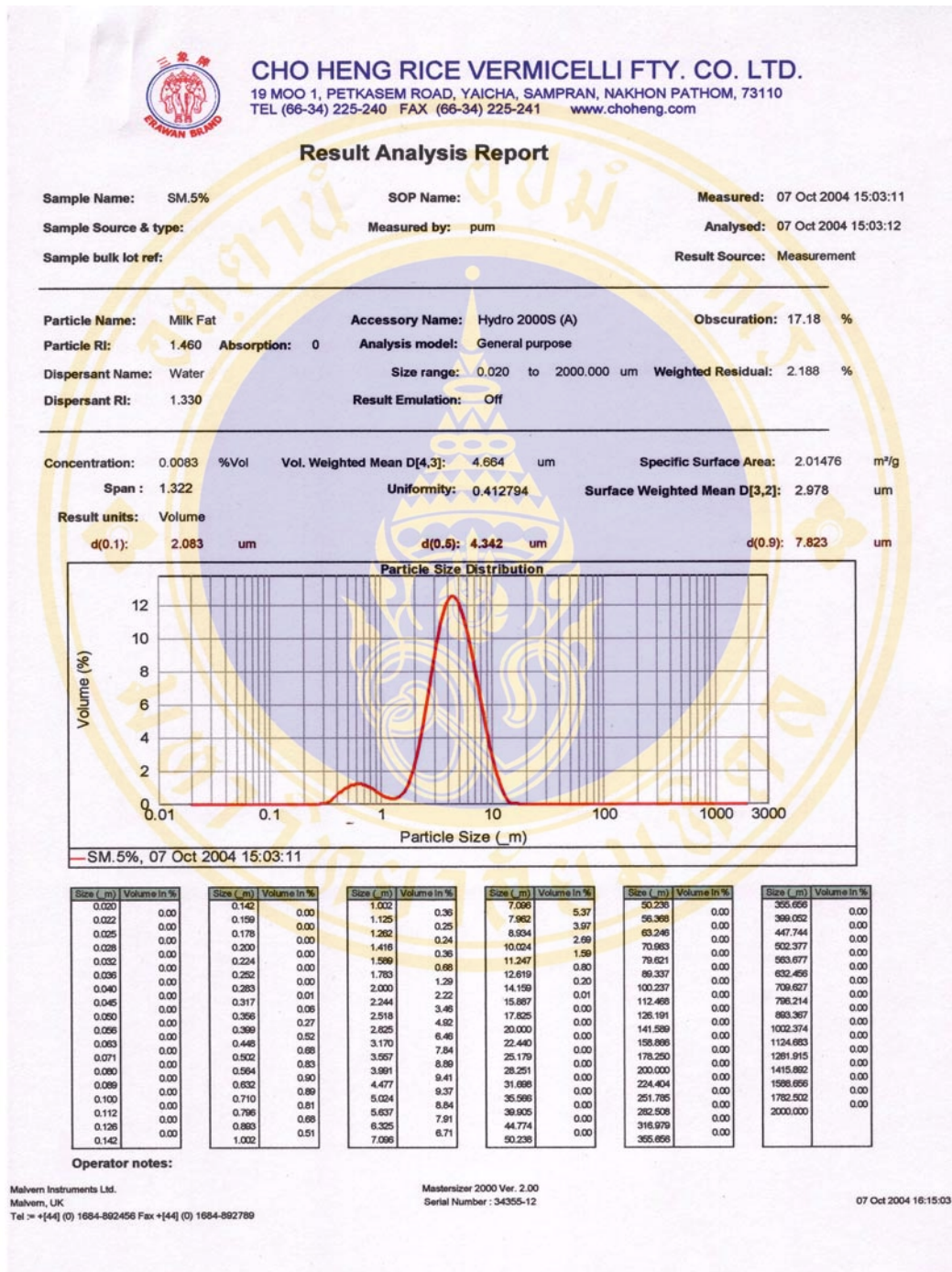


Figure 41 Particle size in raw milk combined with 5% reconstituted SMP

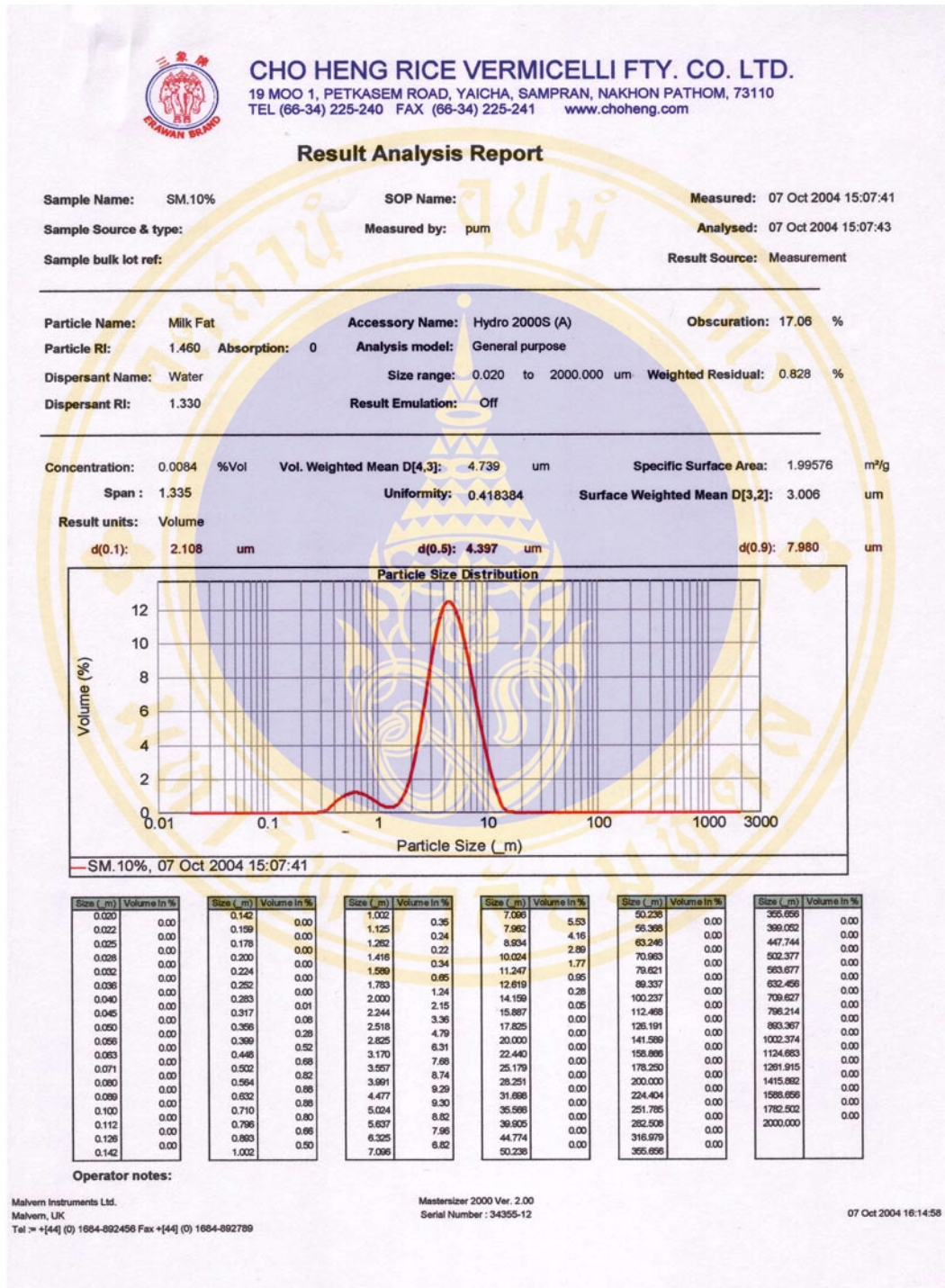


Figure 42 Particle size in raw milk combined with 10% reconstituted SMP

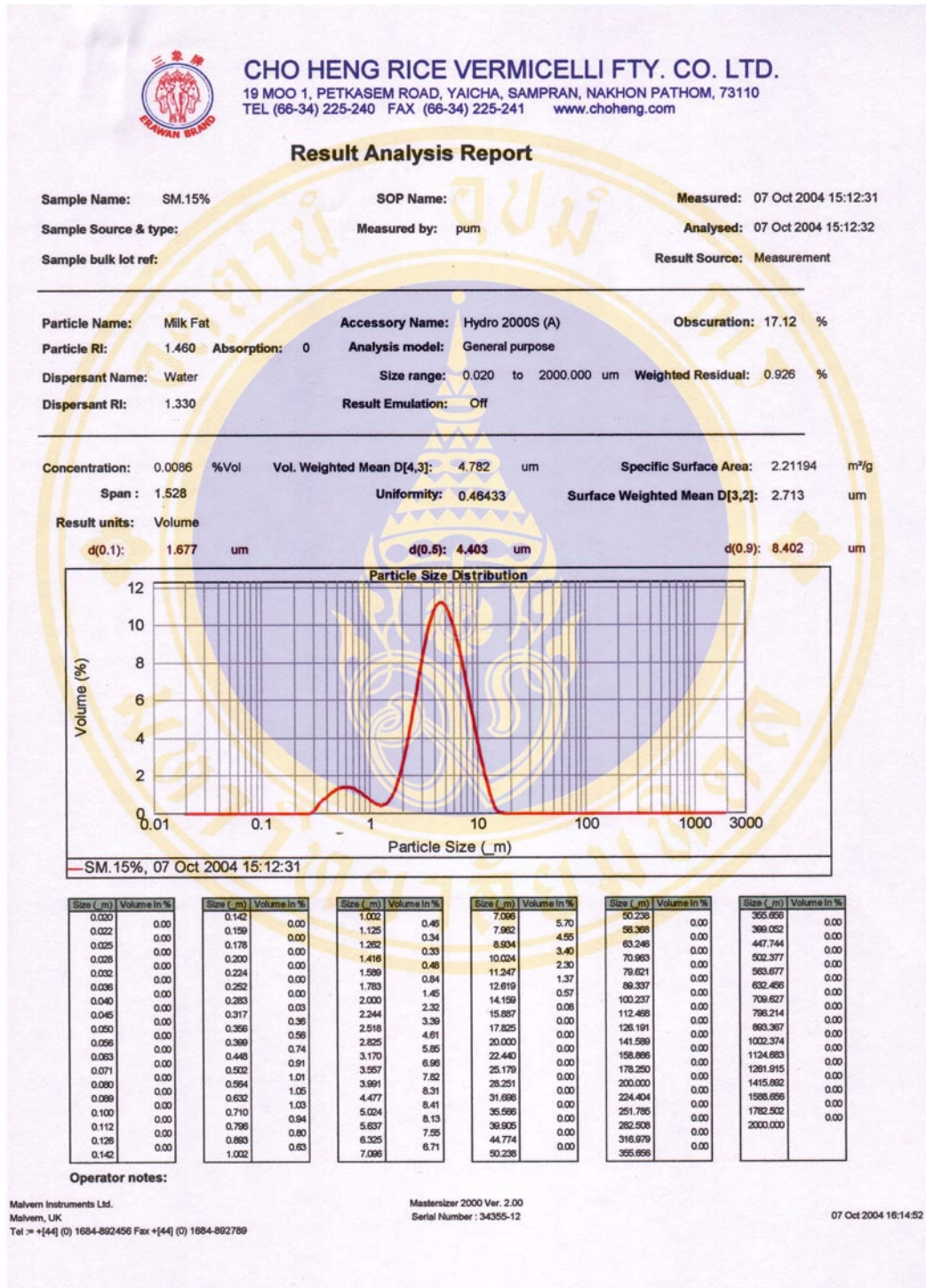


Figure 43 Particle size in raw milk combined with 15% reconstituted SMP

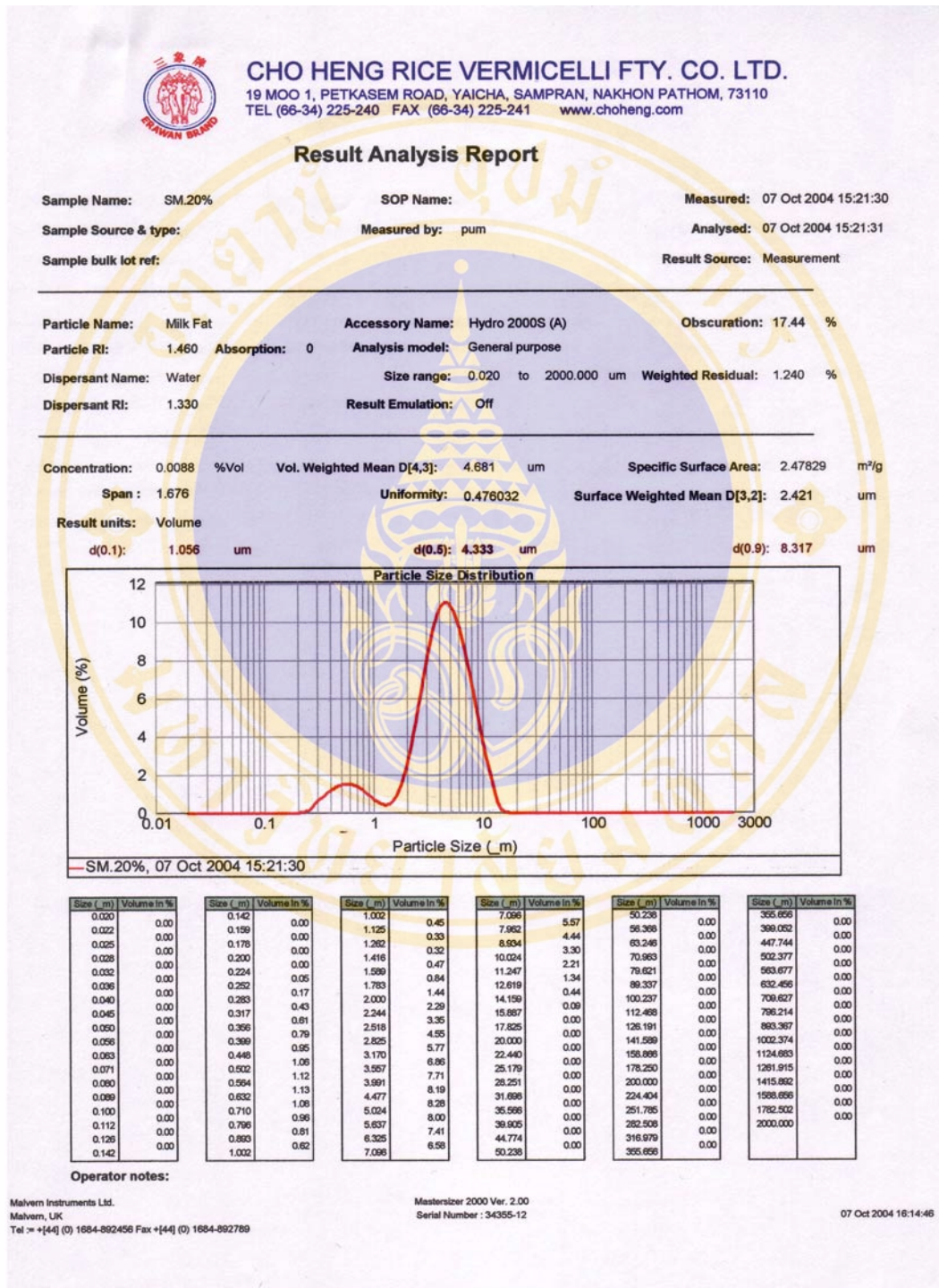


Figure 44 Particle size in raw milk combined with 20% reconstituted SMP

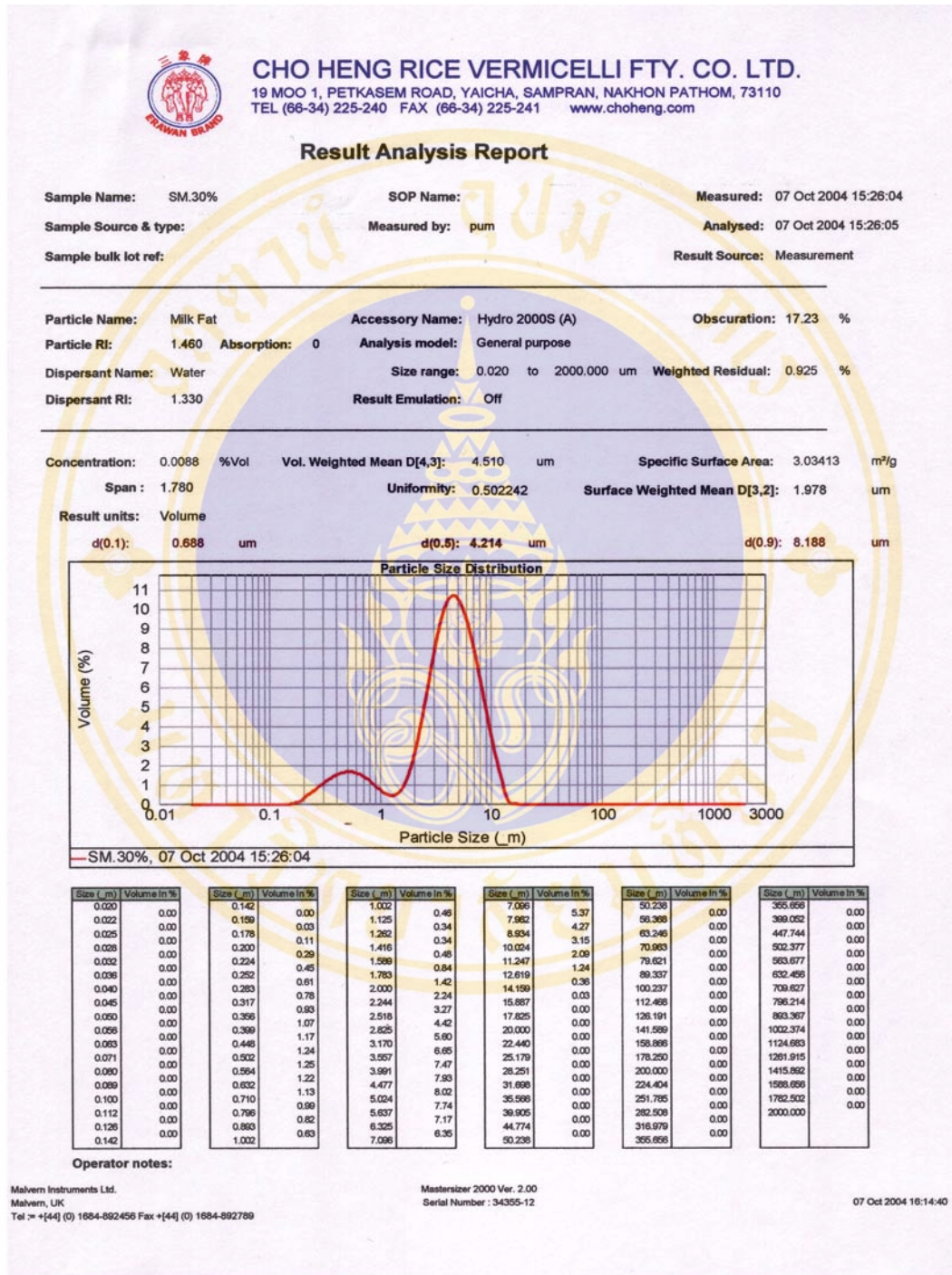


Figure 45 Particle size in raw milk combined with 30% reconstituted SMP

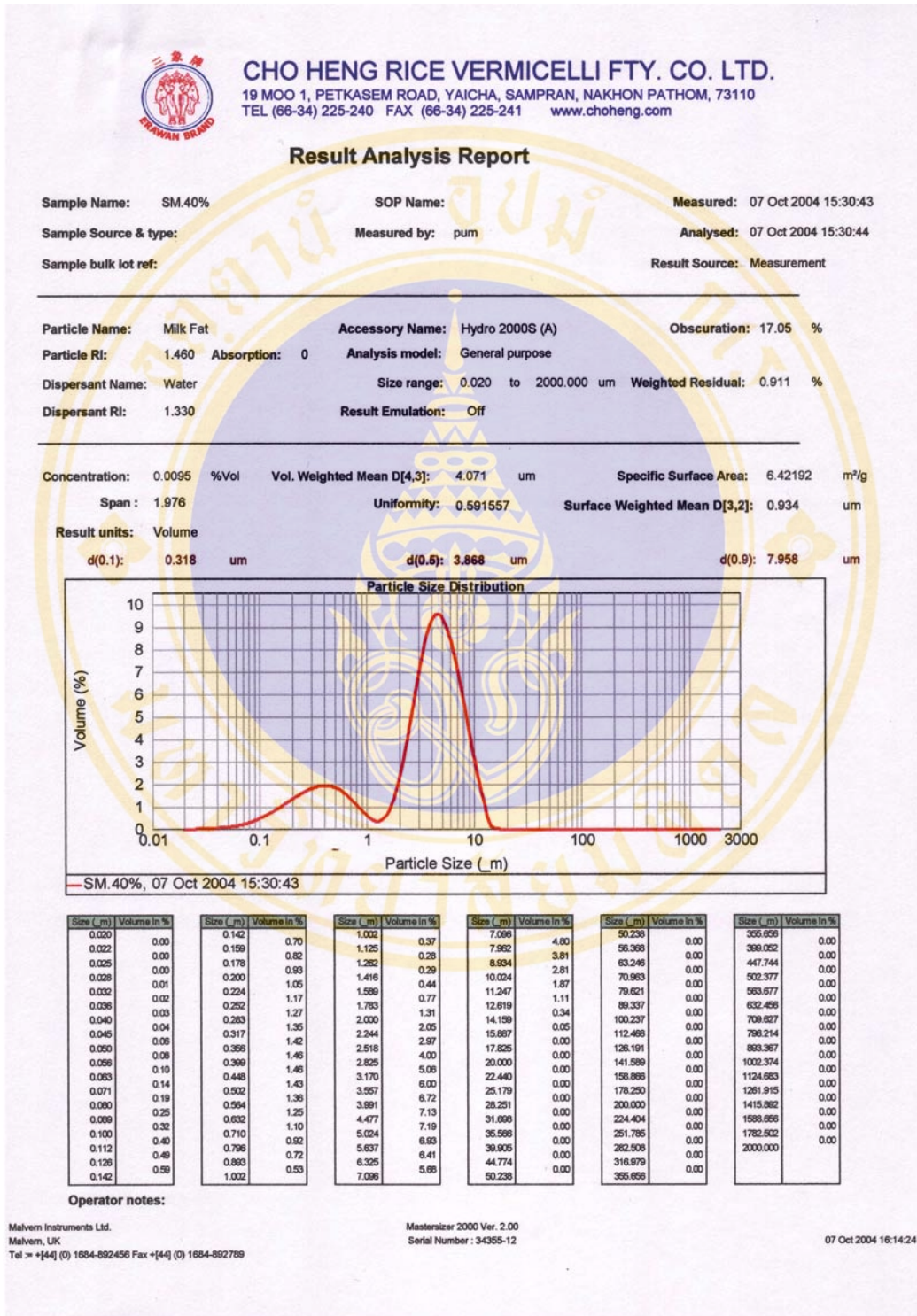


Figure 46 Particle size in raw milk combined with 40% reconstituted SMP

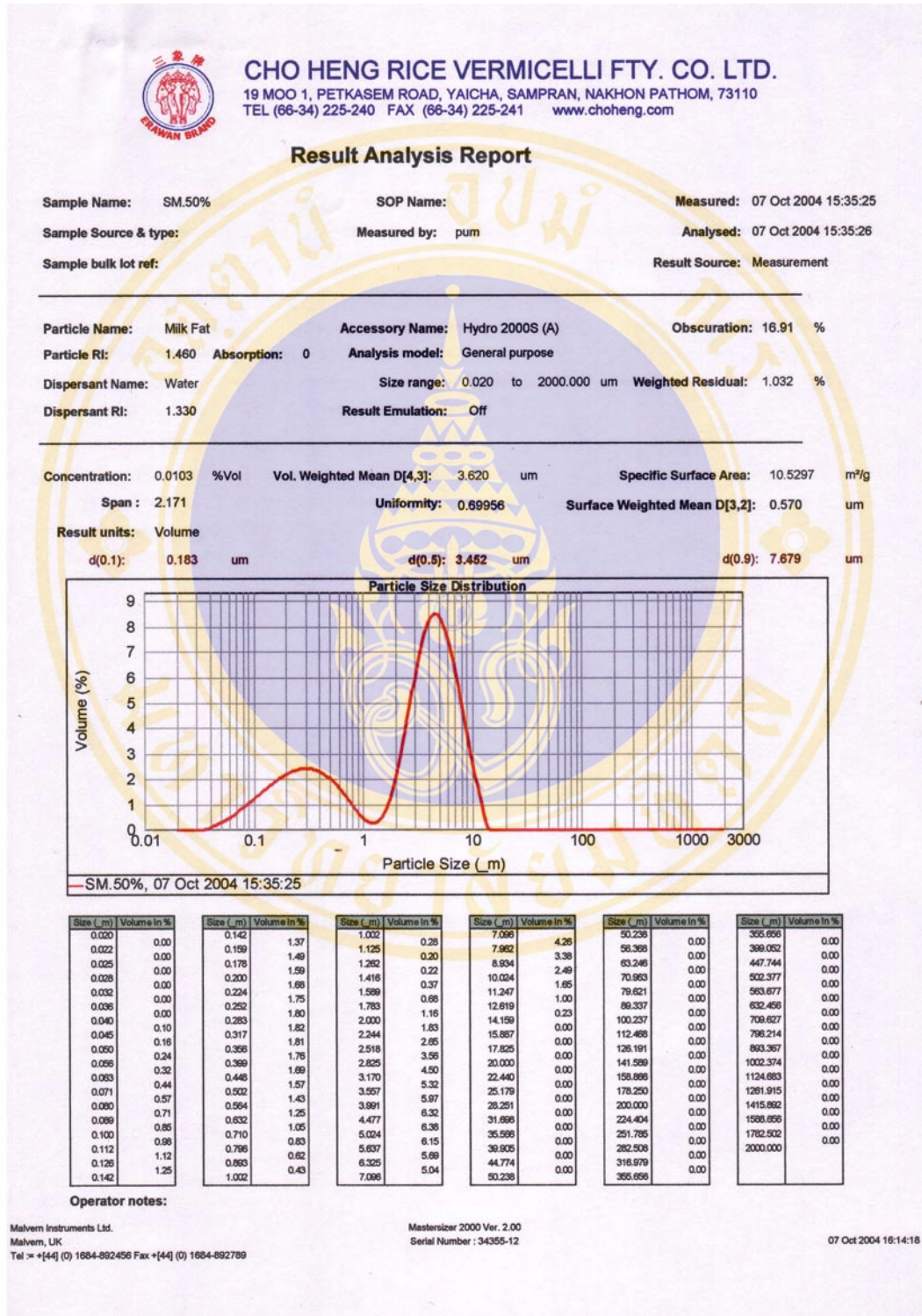


Figure 47 Particle size in raw milk combined with 50% reconstituted SMP

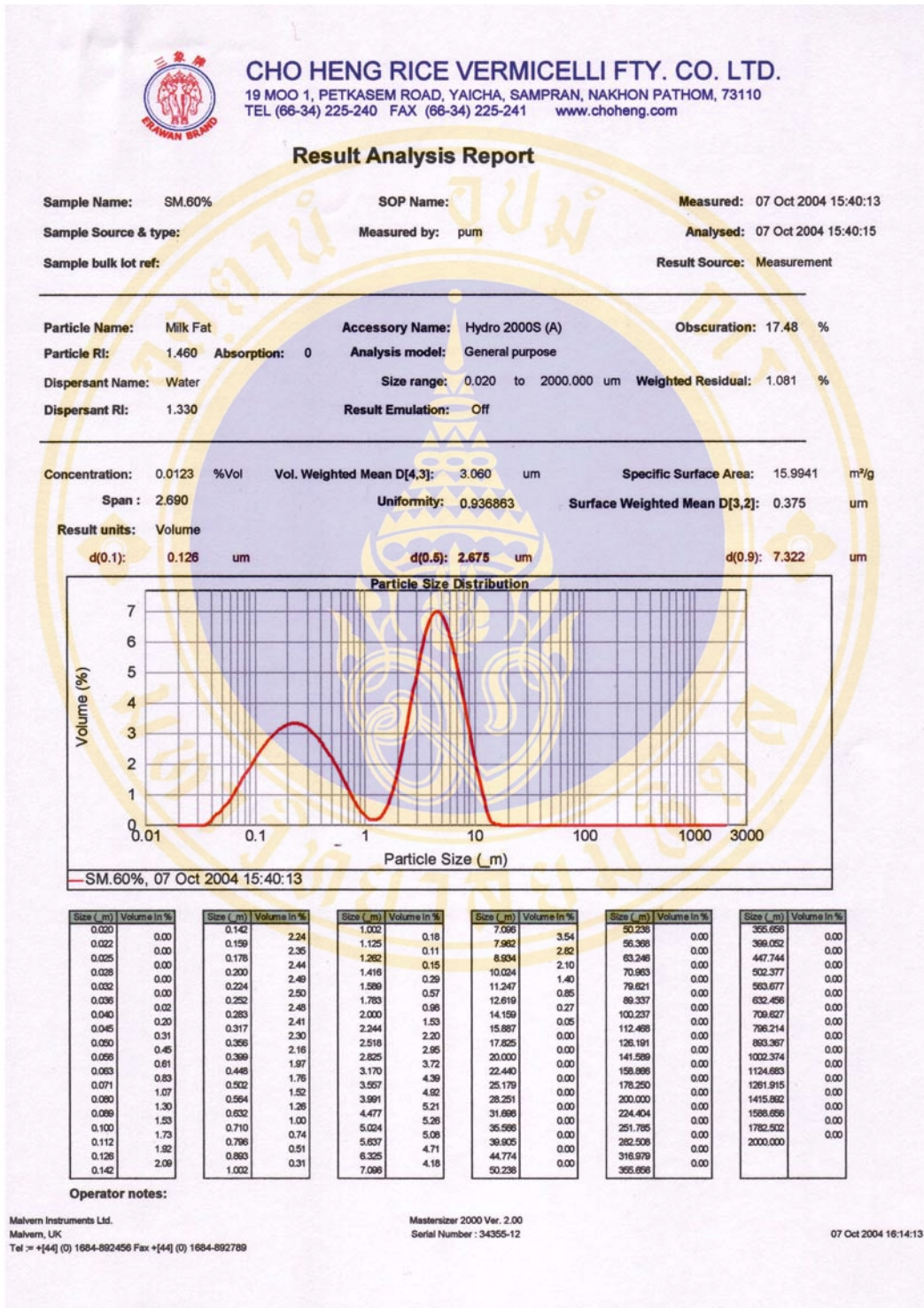


Figure 48 Particle size in raw milk combined with 60% reconstituted SMP

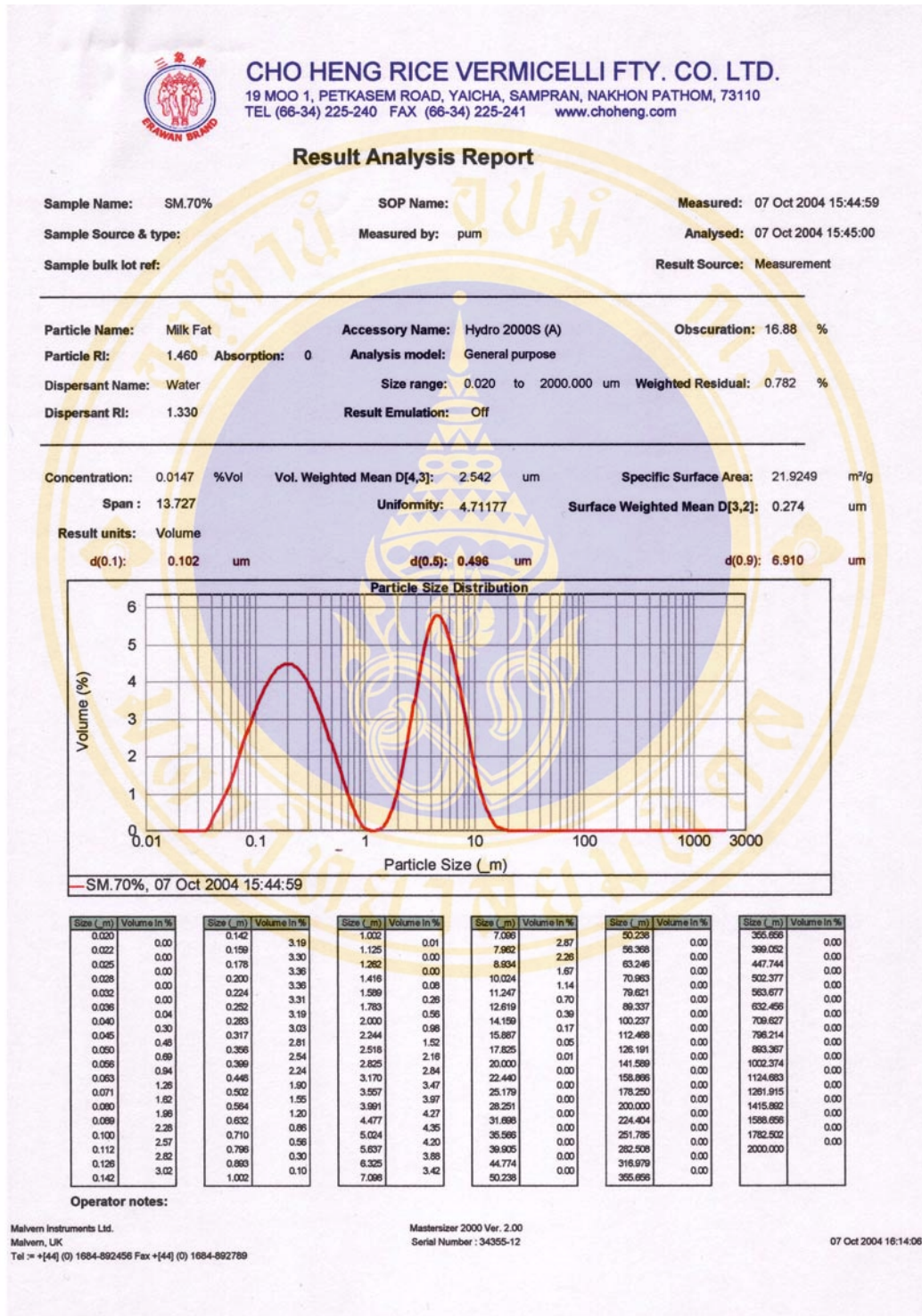


Figure 49 Particle size in raw milk combined with 70% reconstituted SMP

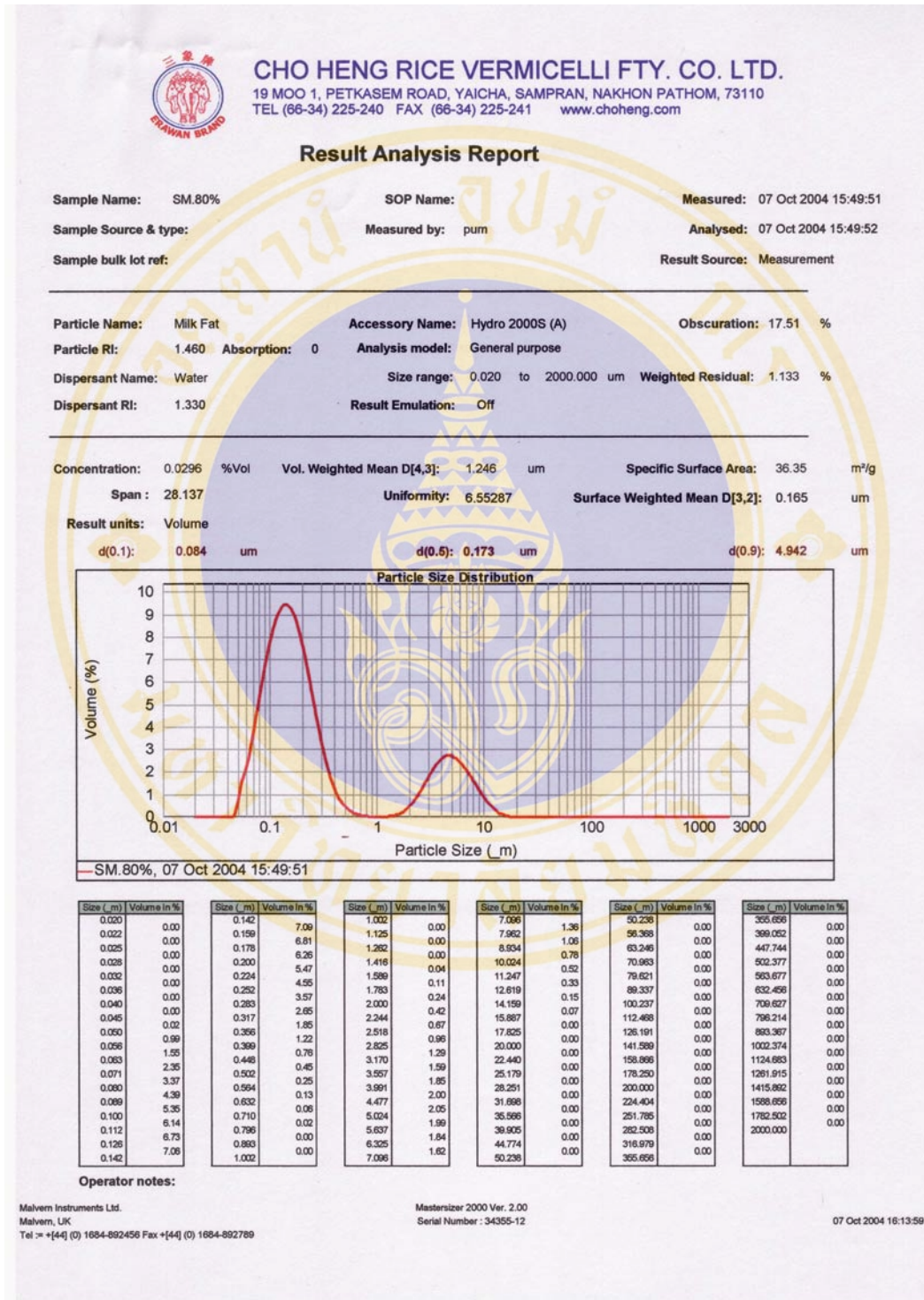


Figure 50 Particle size in raw milk combined with 80% reconstituted SMP

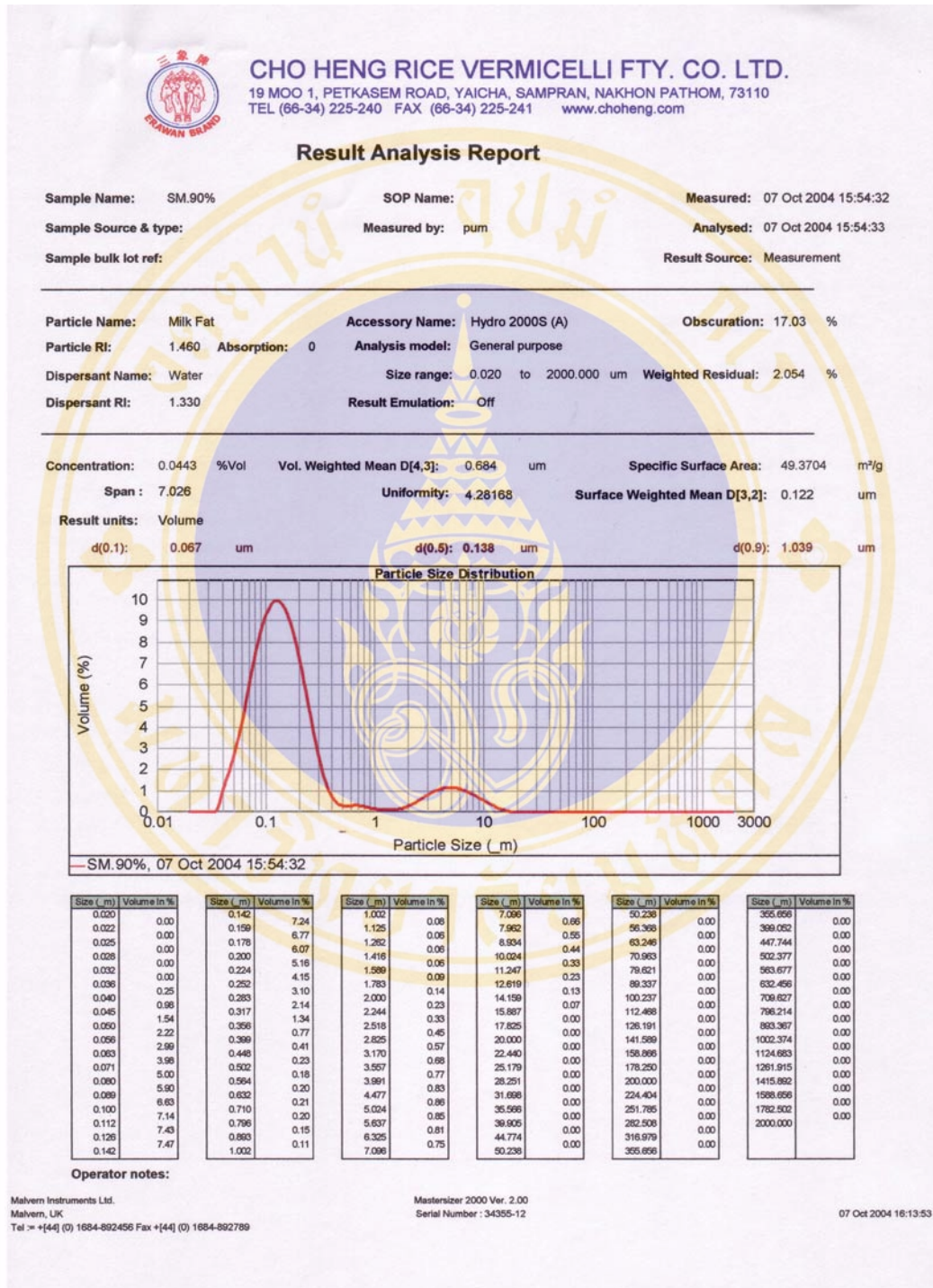


Figure 51 Particle size in raw milk combined with 90% reconstituted SMP

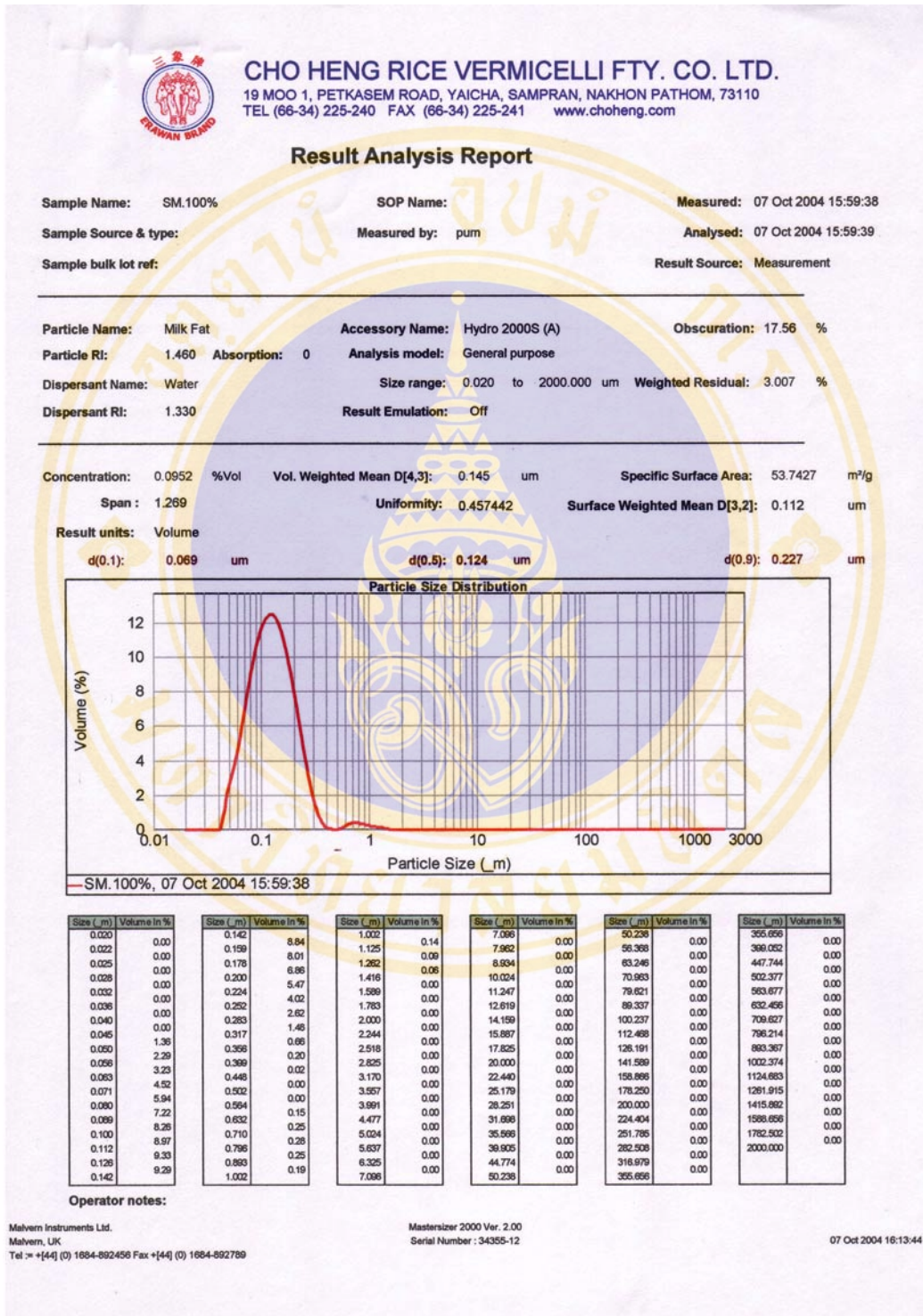


Figure 52 Particle size in raw milk combined with 100% reconstituted SMP

### 3. Particle size in pasteurized milk purchased from supermarkets in Bangkok

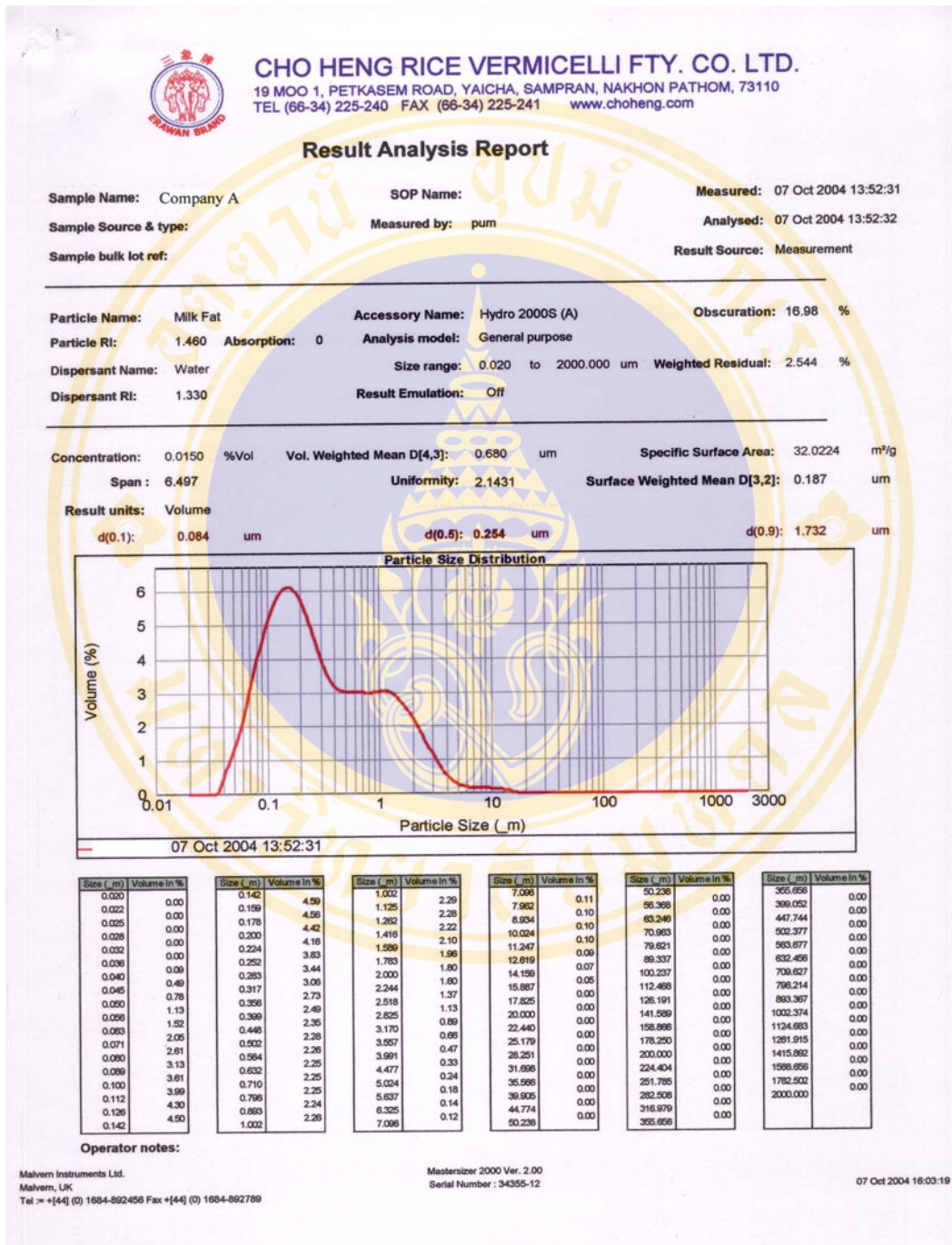


Figure 53 Particle size in pasteurized milk of company A

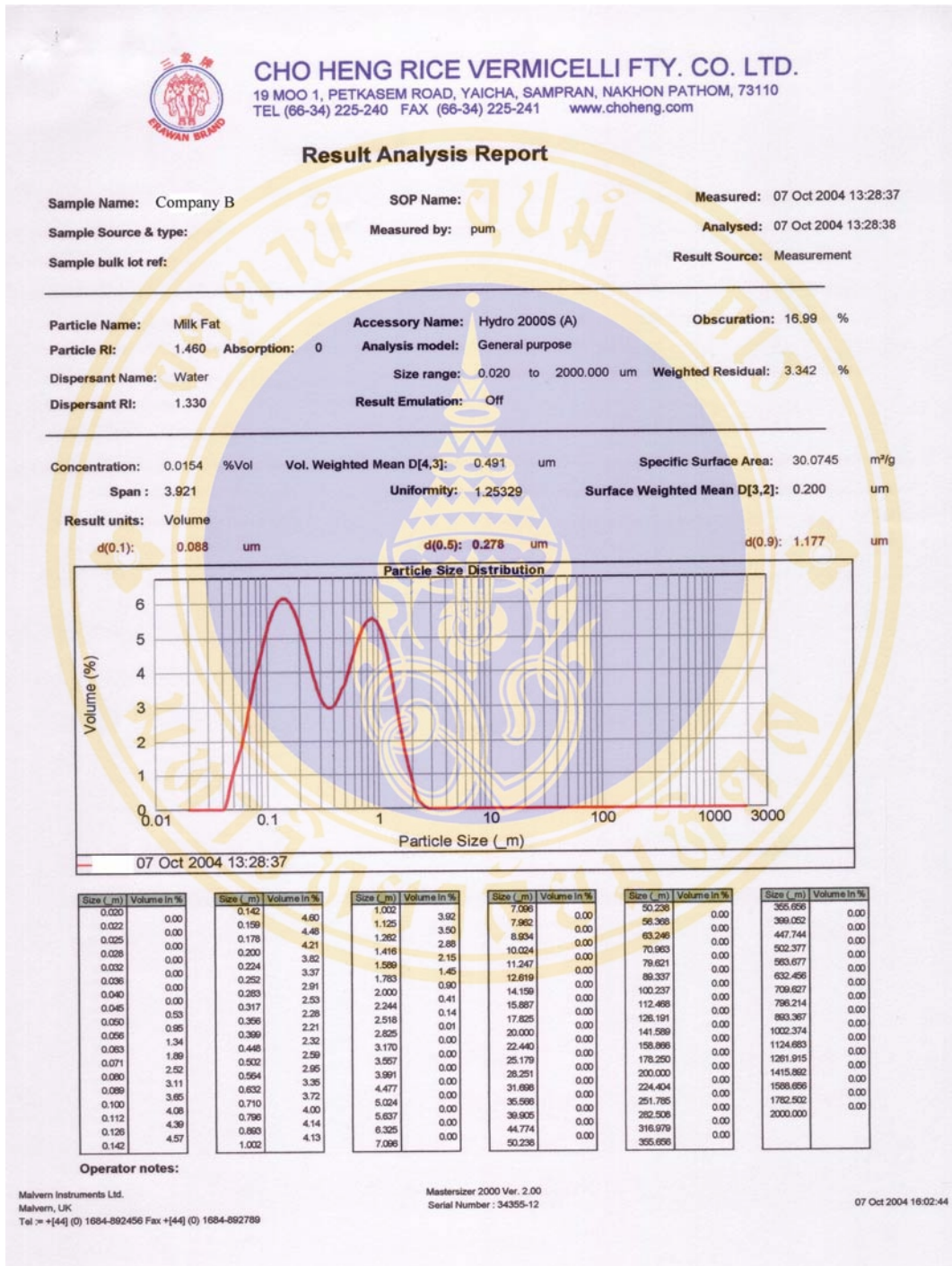


Figure 54 Particle size in pasteurized milk of company B

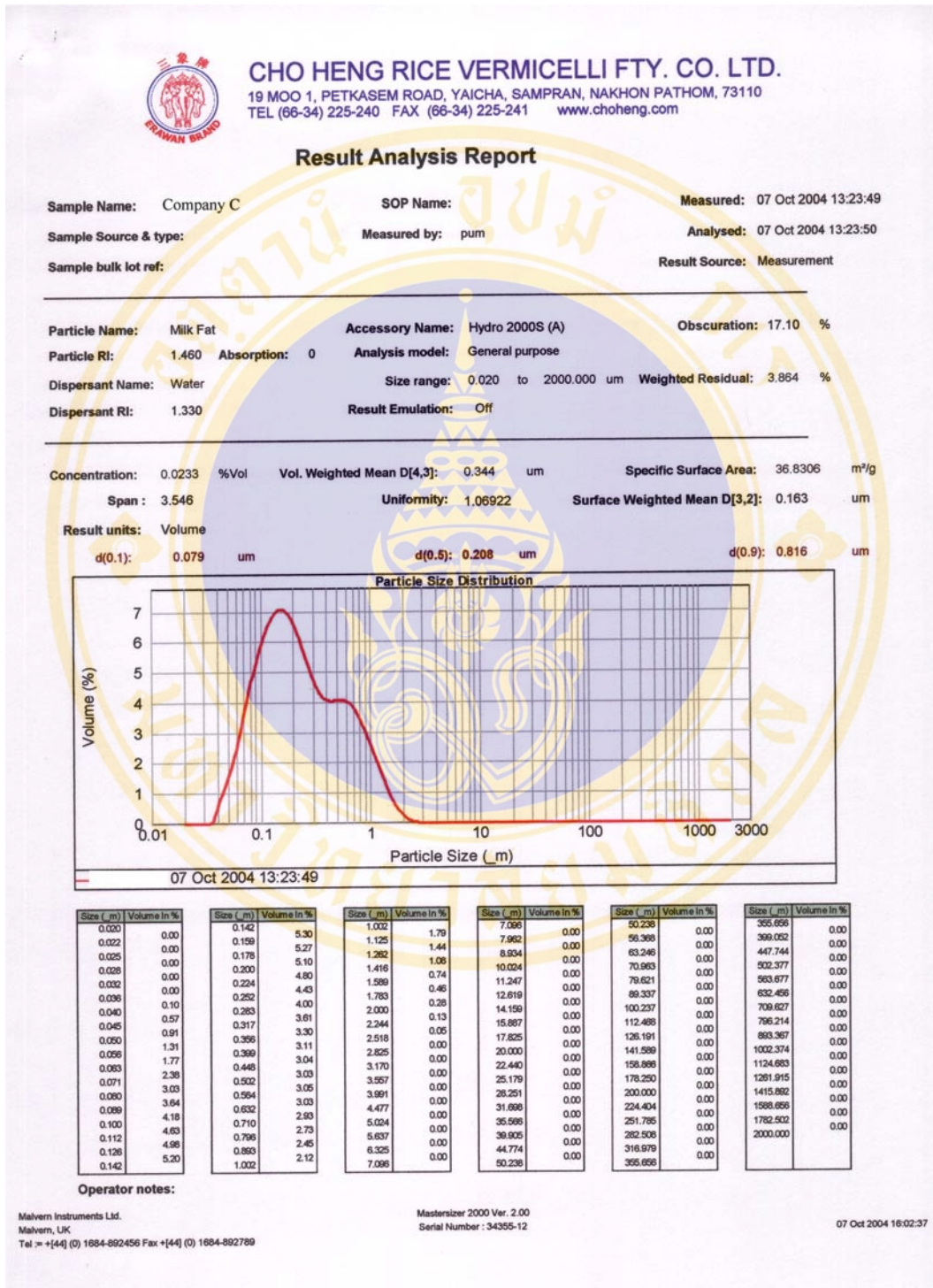


Figure 55 Particle size in pasteurized milk of company C

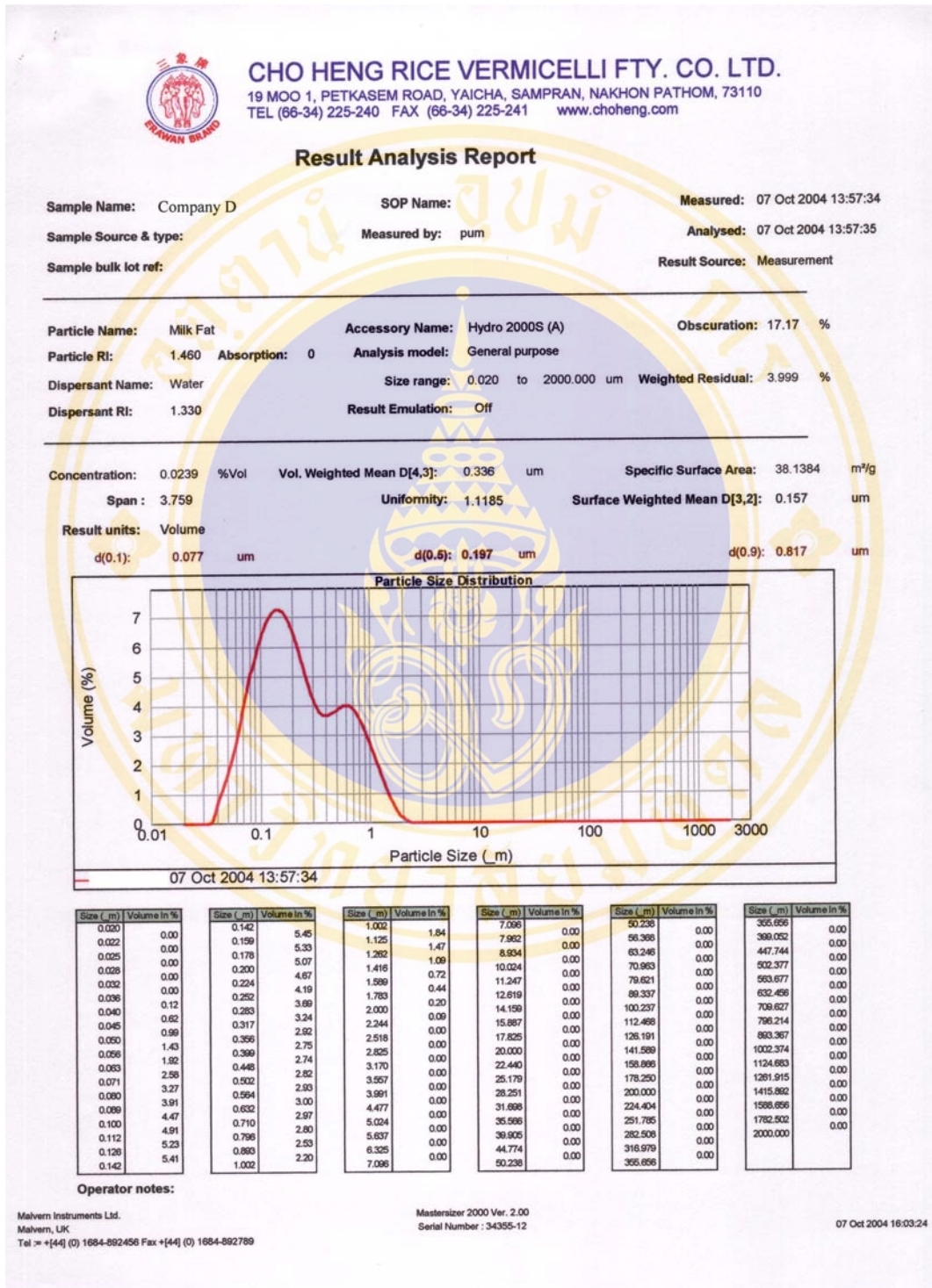


Figure 56 Particle size in pasteurized milk of company D

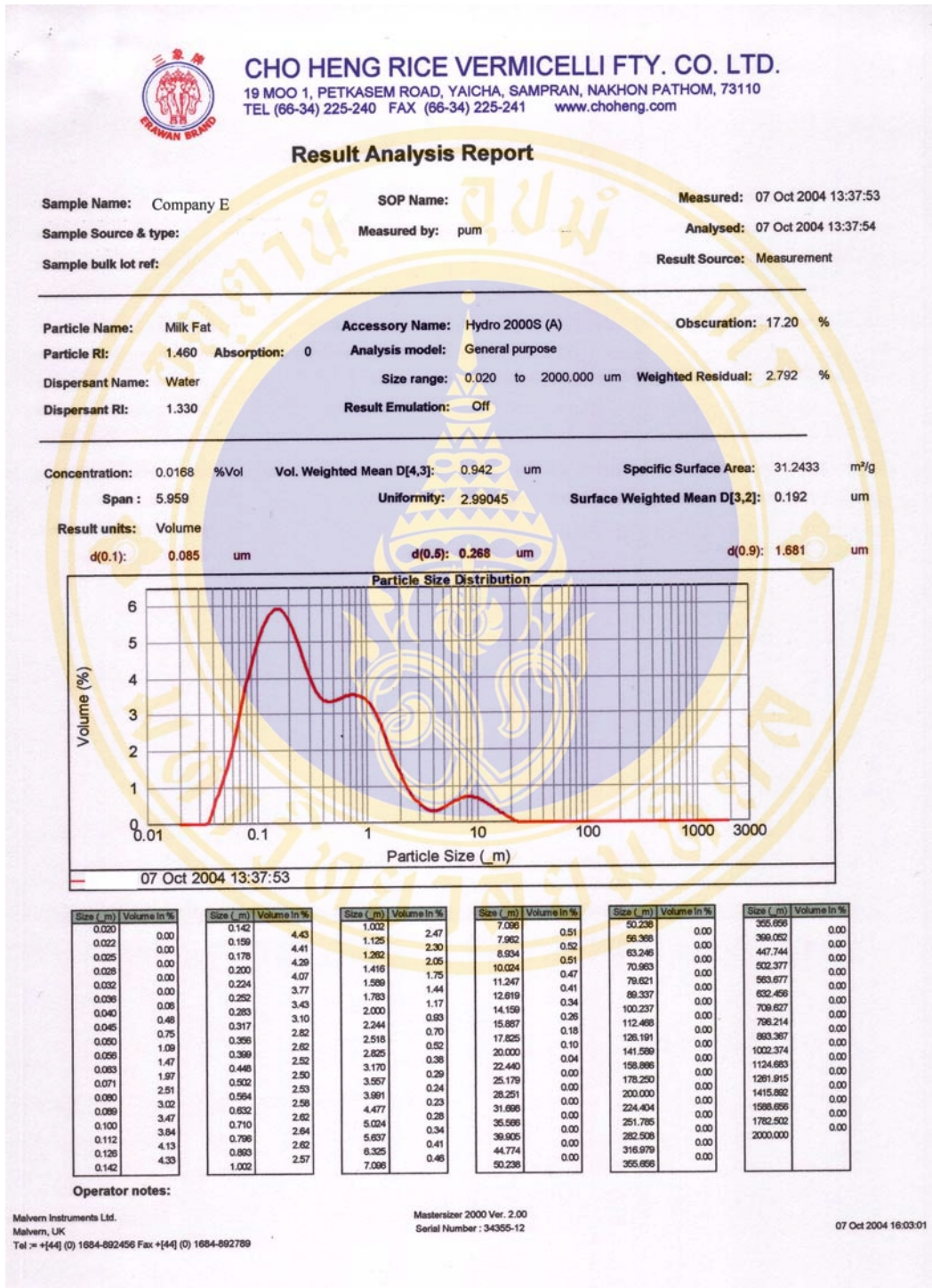


Figure 57 Particle size in pasteurized milk of company E

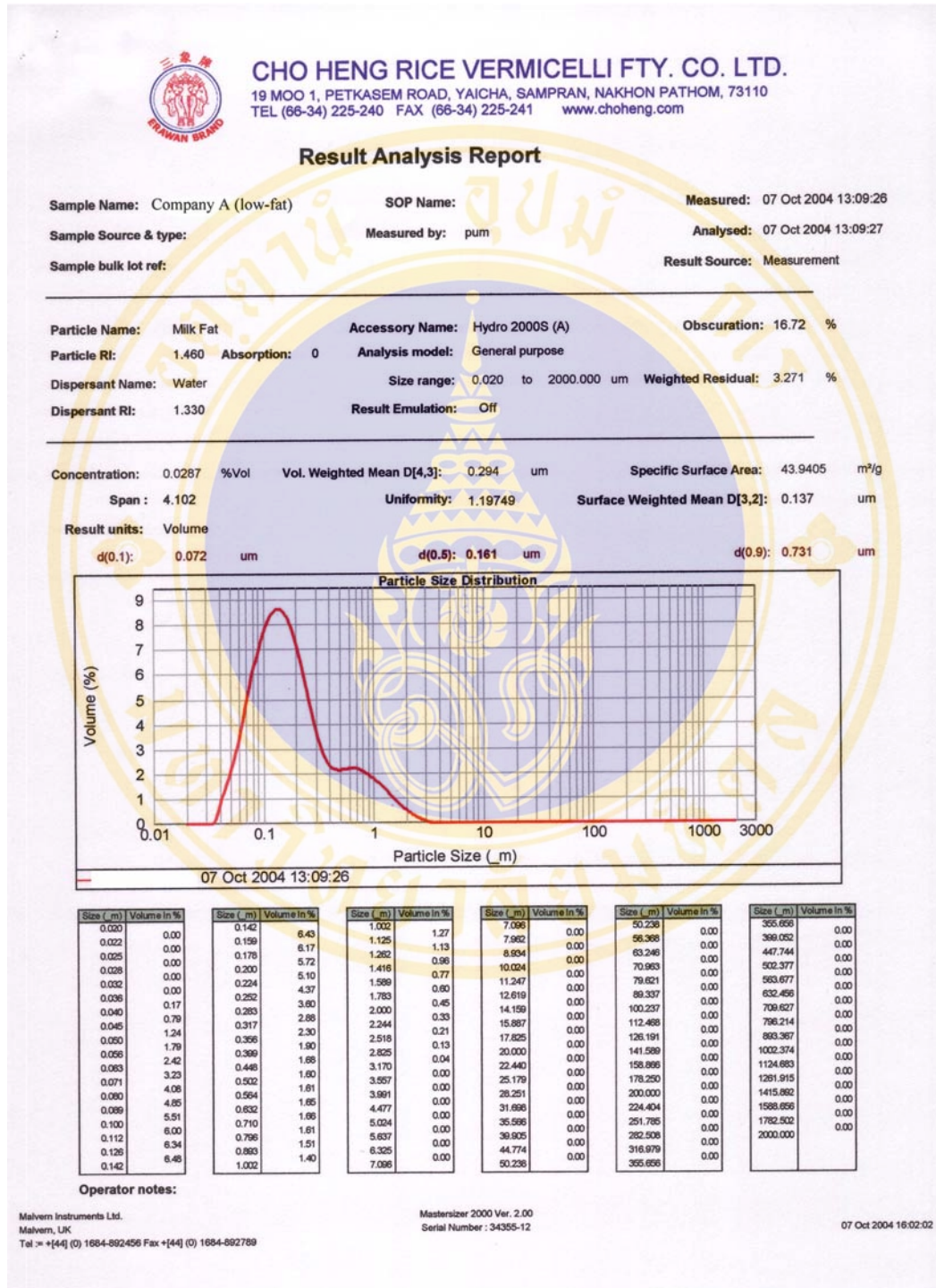


Figure 58 Particle size in pasteurized low-fat milk of company A

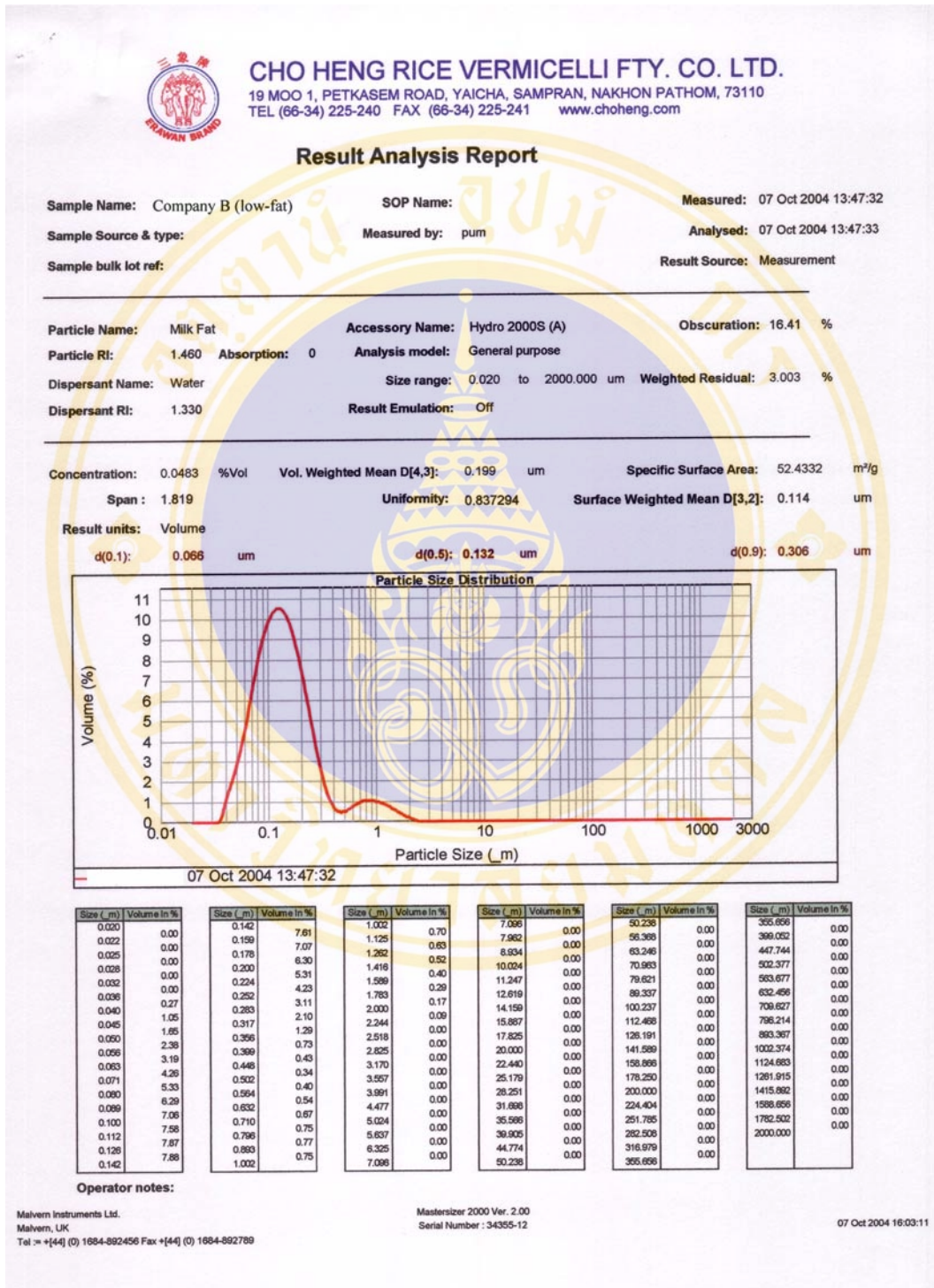


Figure 59 Particle size in pasteurized low-fat milk of company B

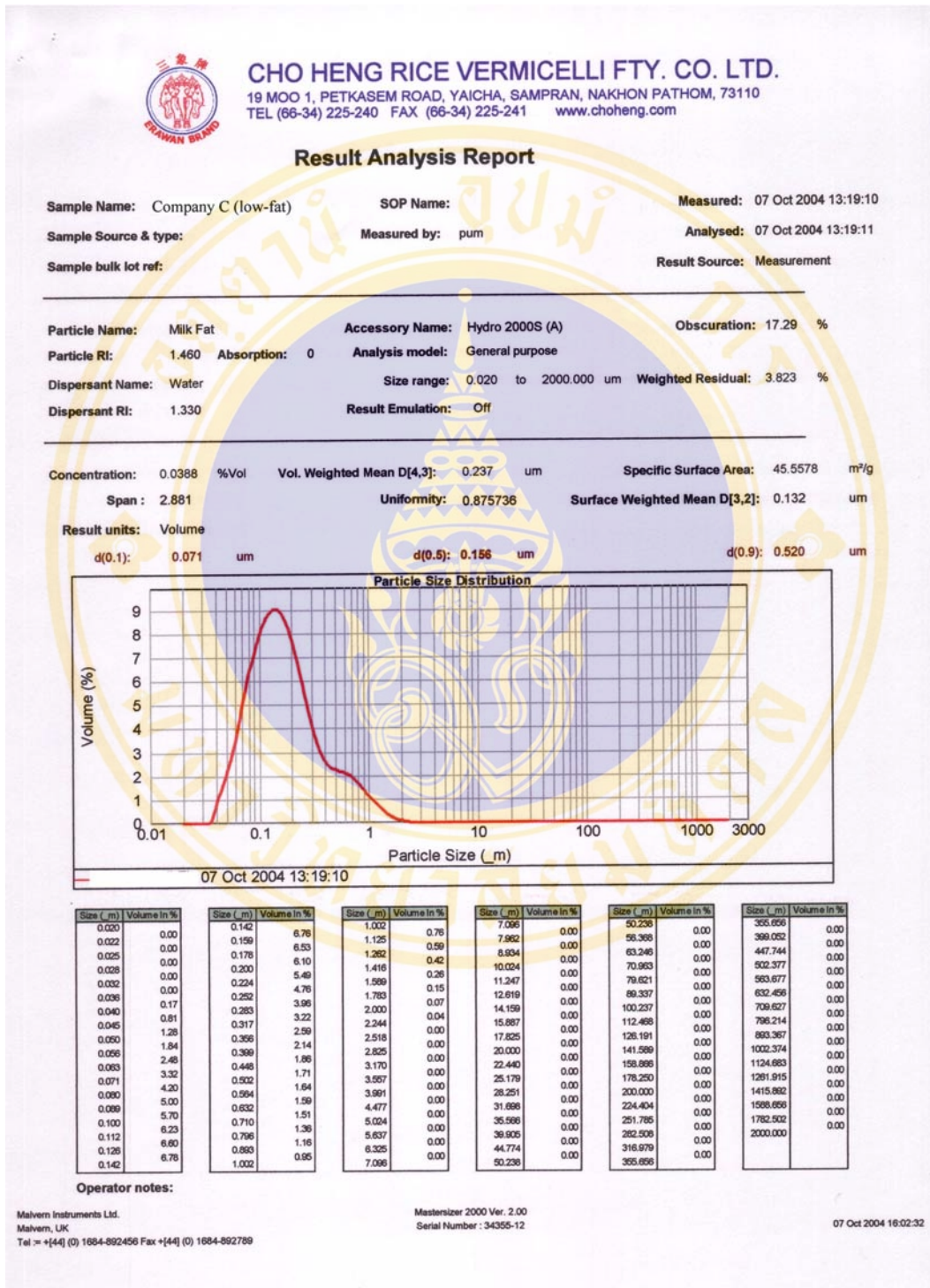


Figure 60 Particle size in pasteurized low-fat milk of company C

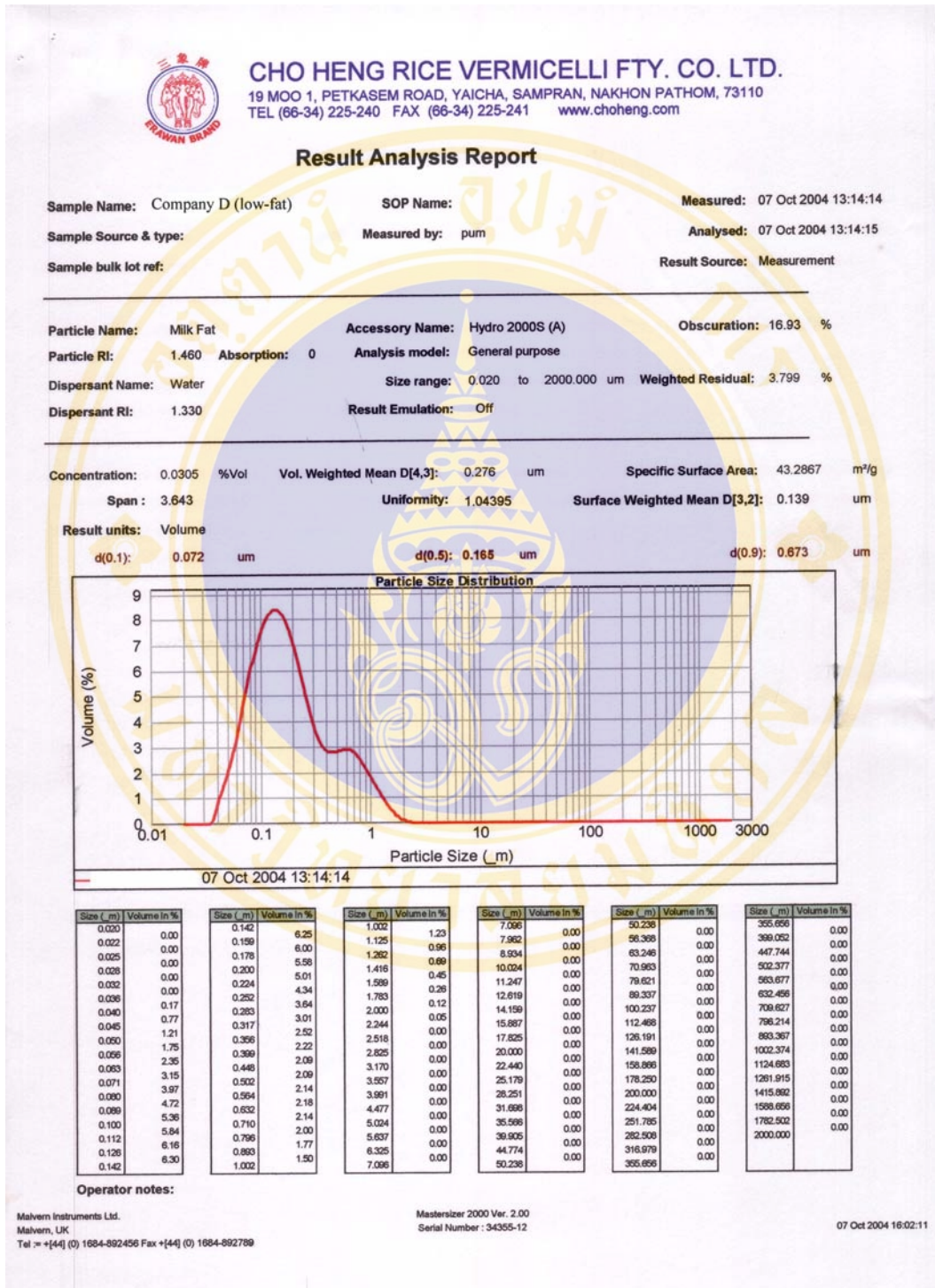


Figure 61 Particle size in pasteurized low-fat milk of company D

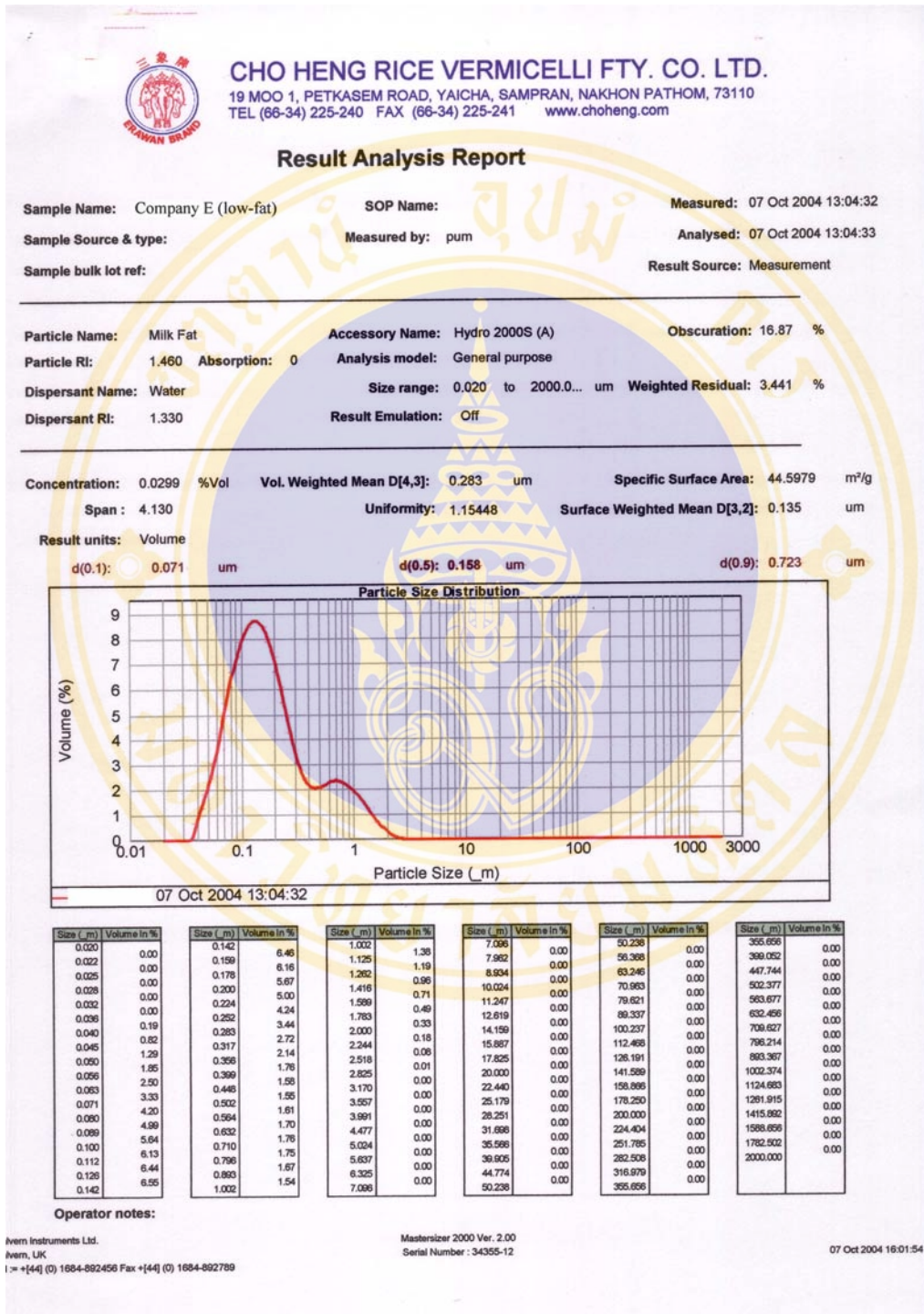


Figure 62 Particle size in pasteurized low-fat milk of company E

**4. Particle size in UHT milk samples purchased from supermarket in Bangkok**

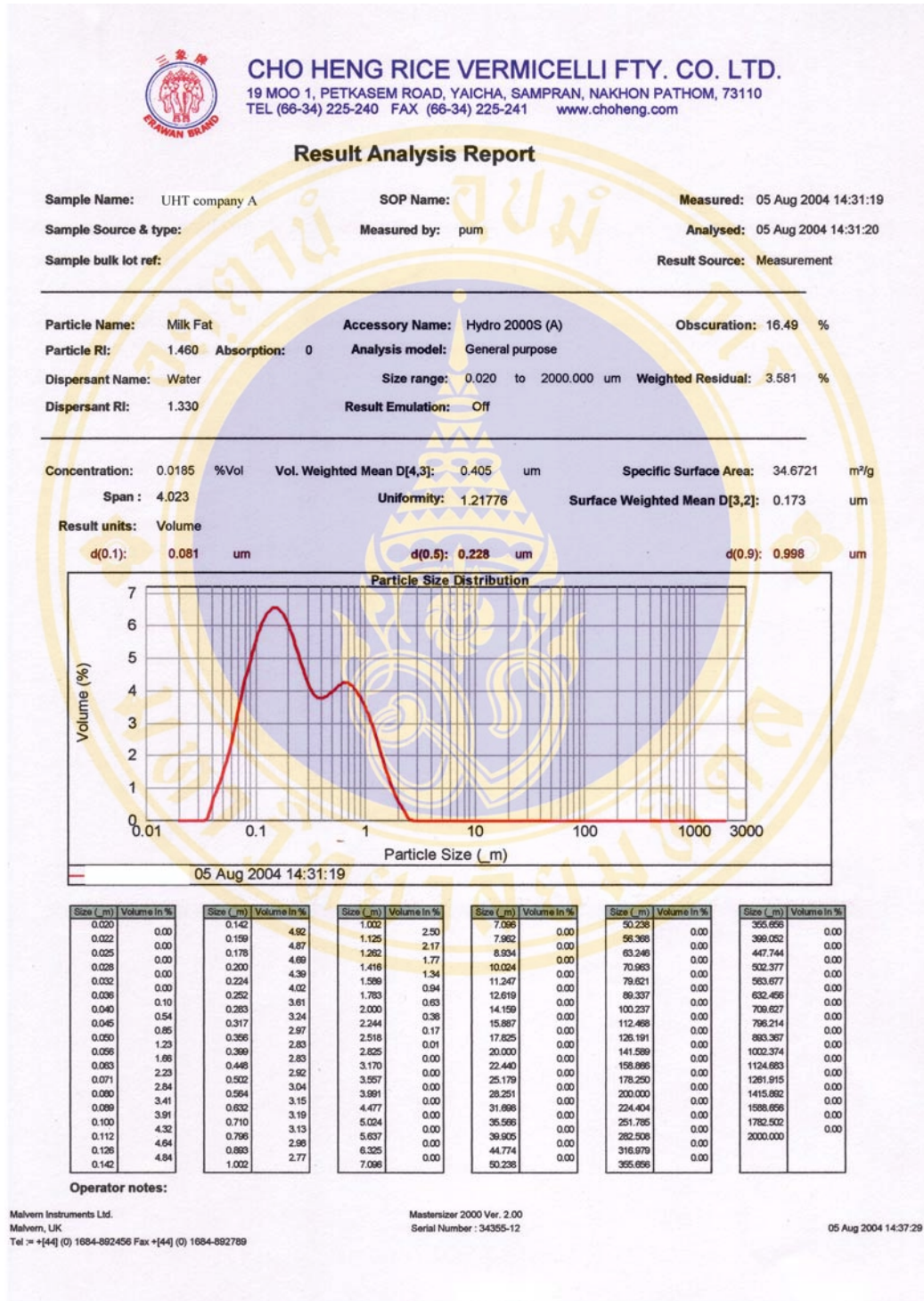


Figure 63 Particle size in UHT milk of company A

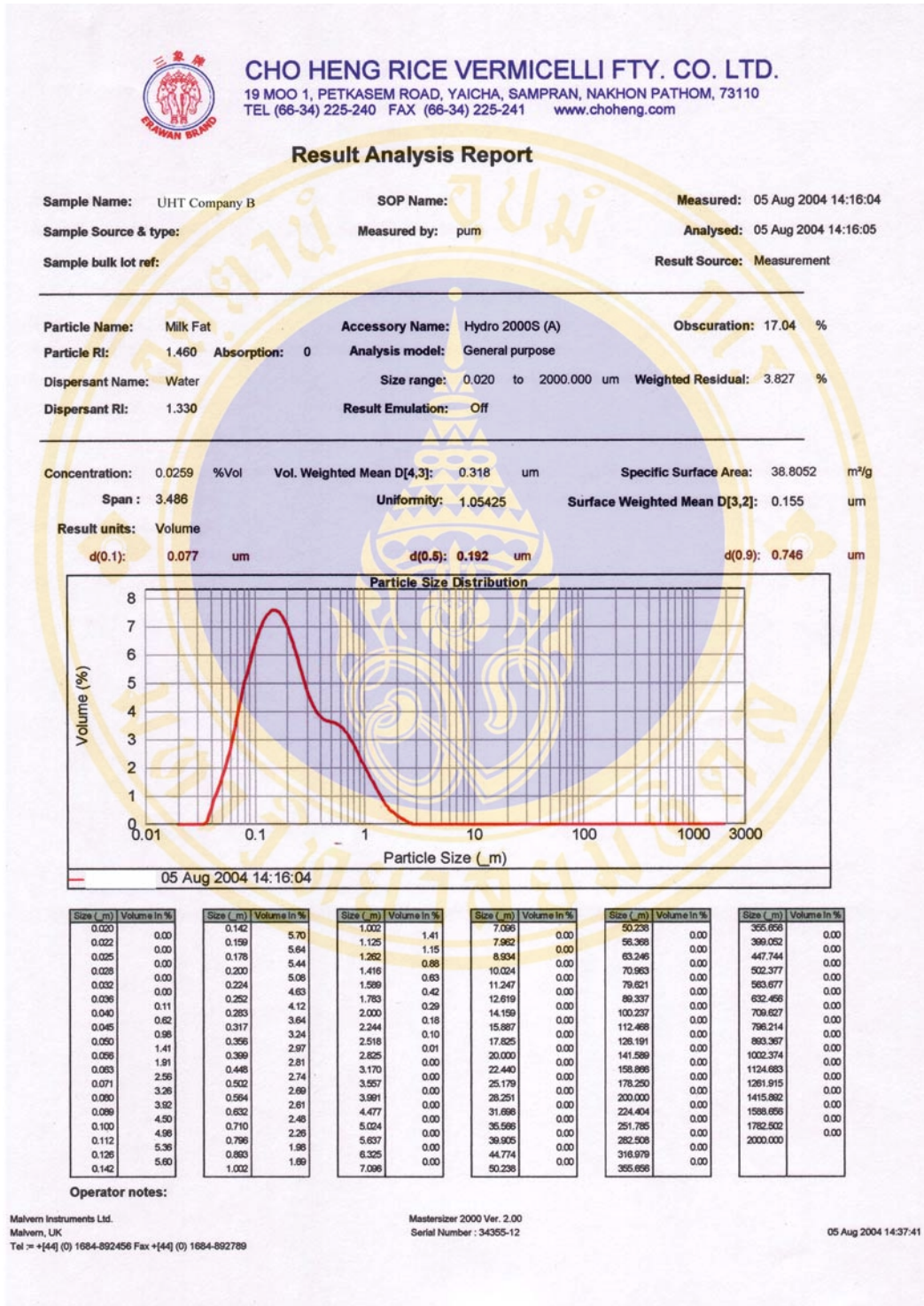


Figure 64 Particle size in UHT milk of company B

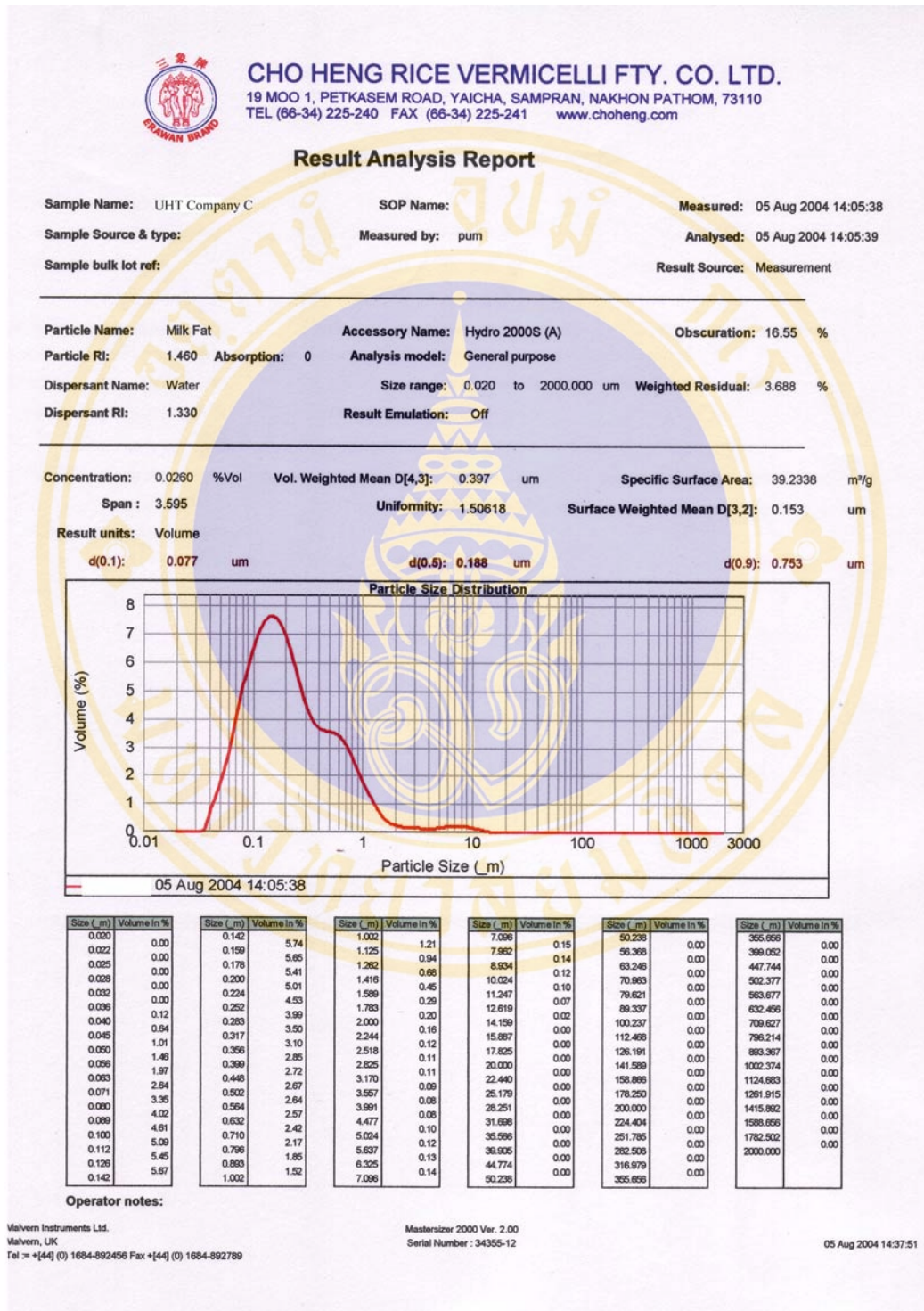


Figure 65 Particle size in UHT milk of company C

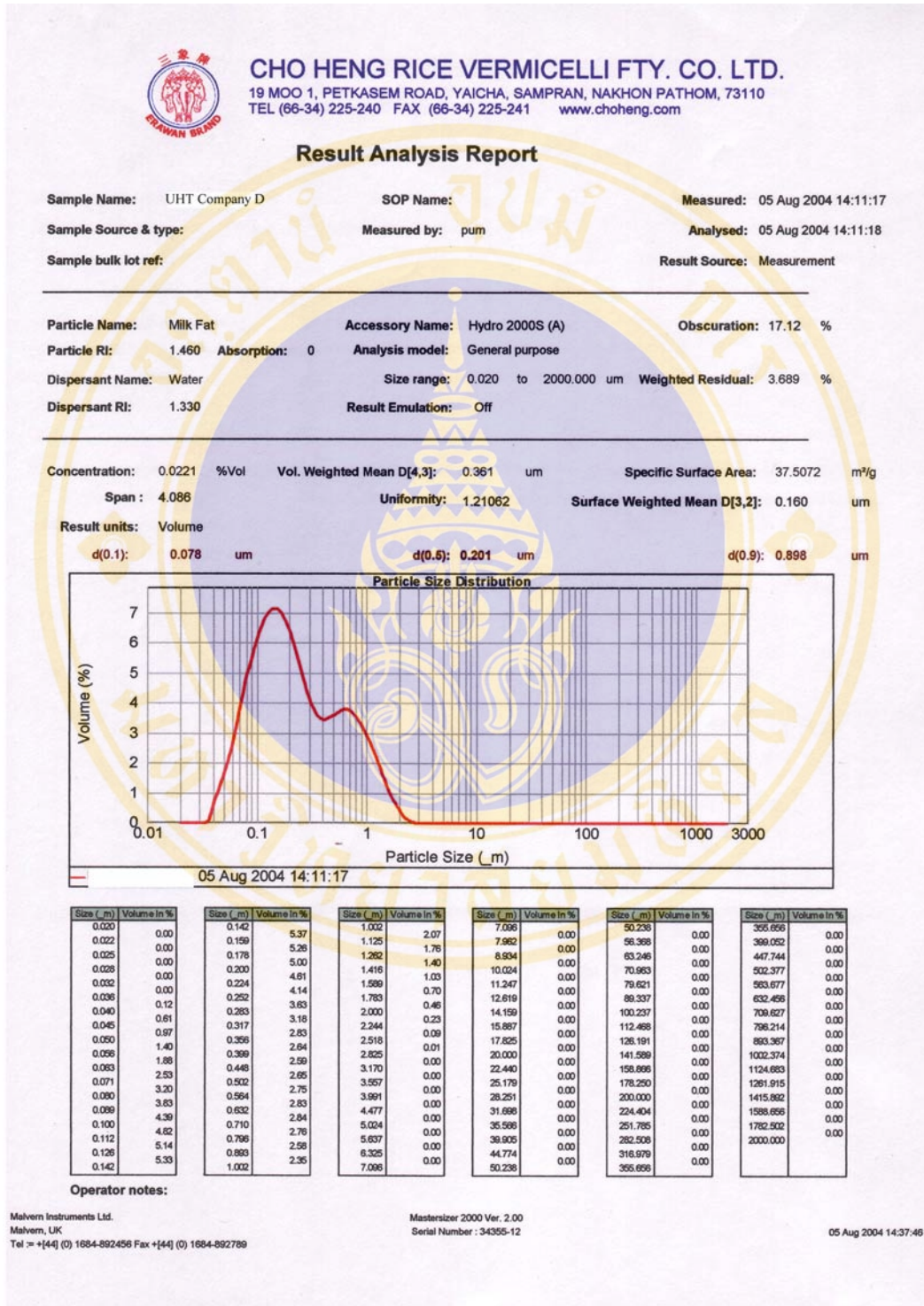


Figure 66 Particle size in UHT milk of company D

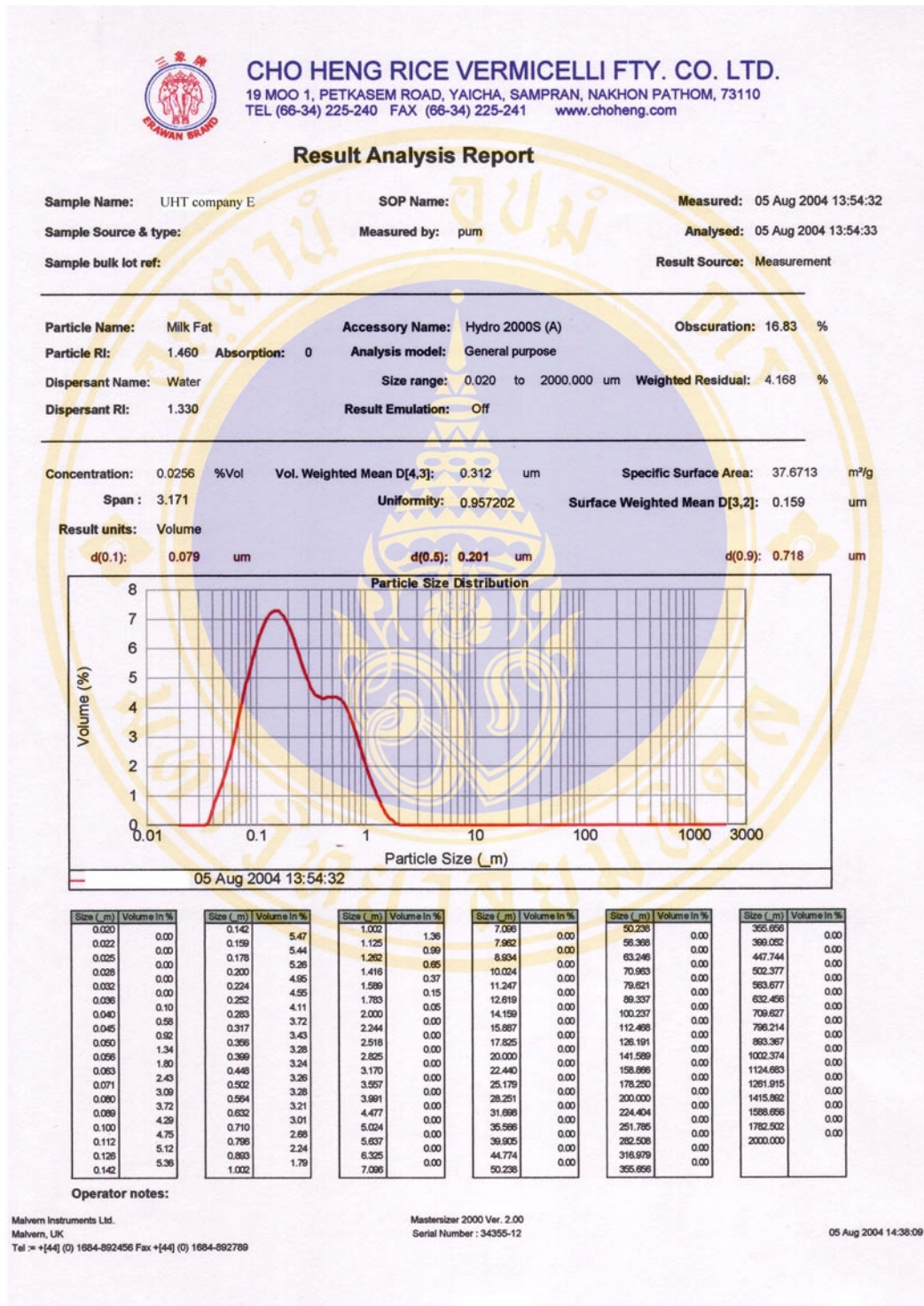


Figure 67 Particle size in UHT milk of company E

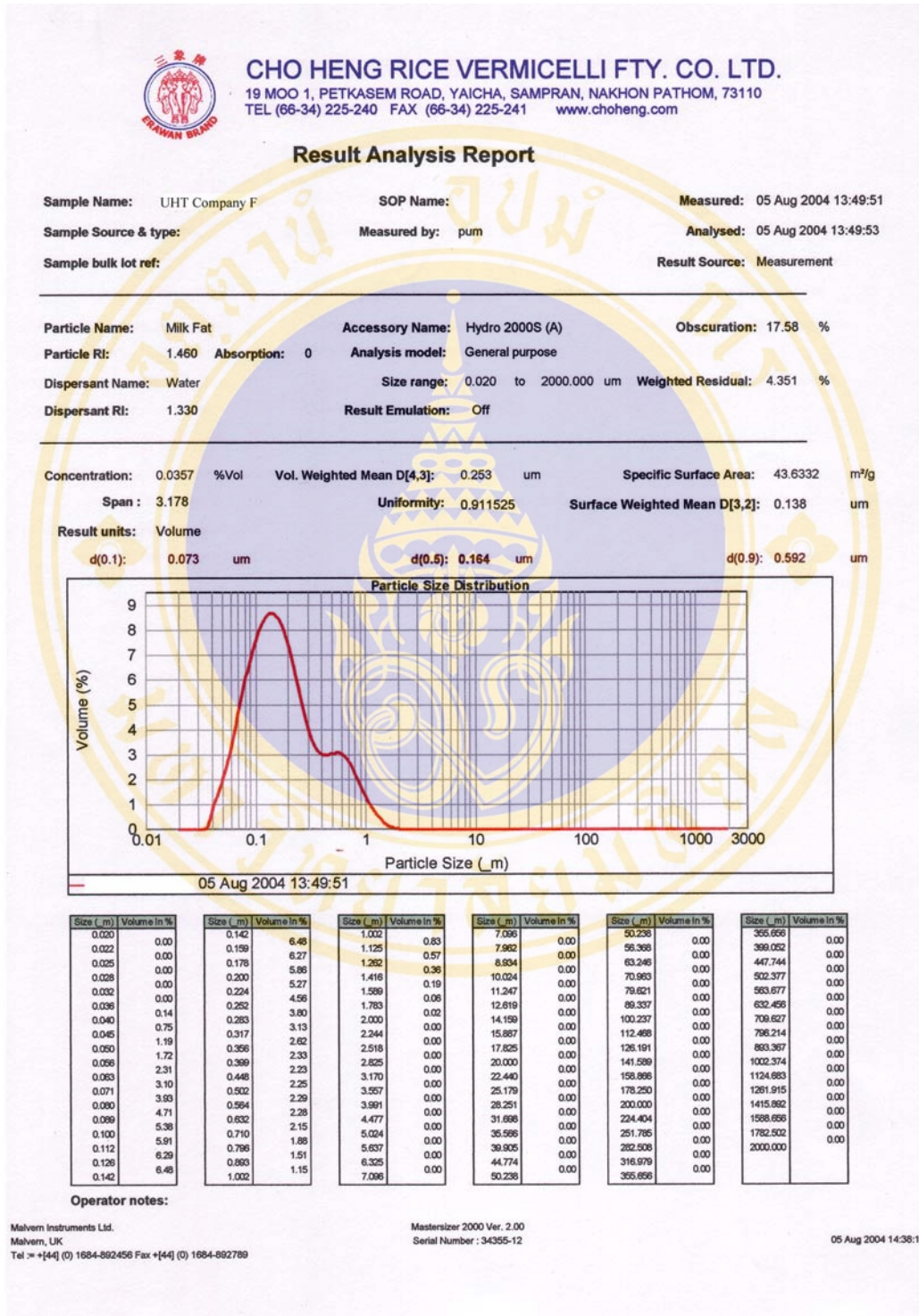


Figure 68 Particle size in UHT milk of company F

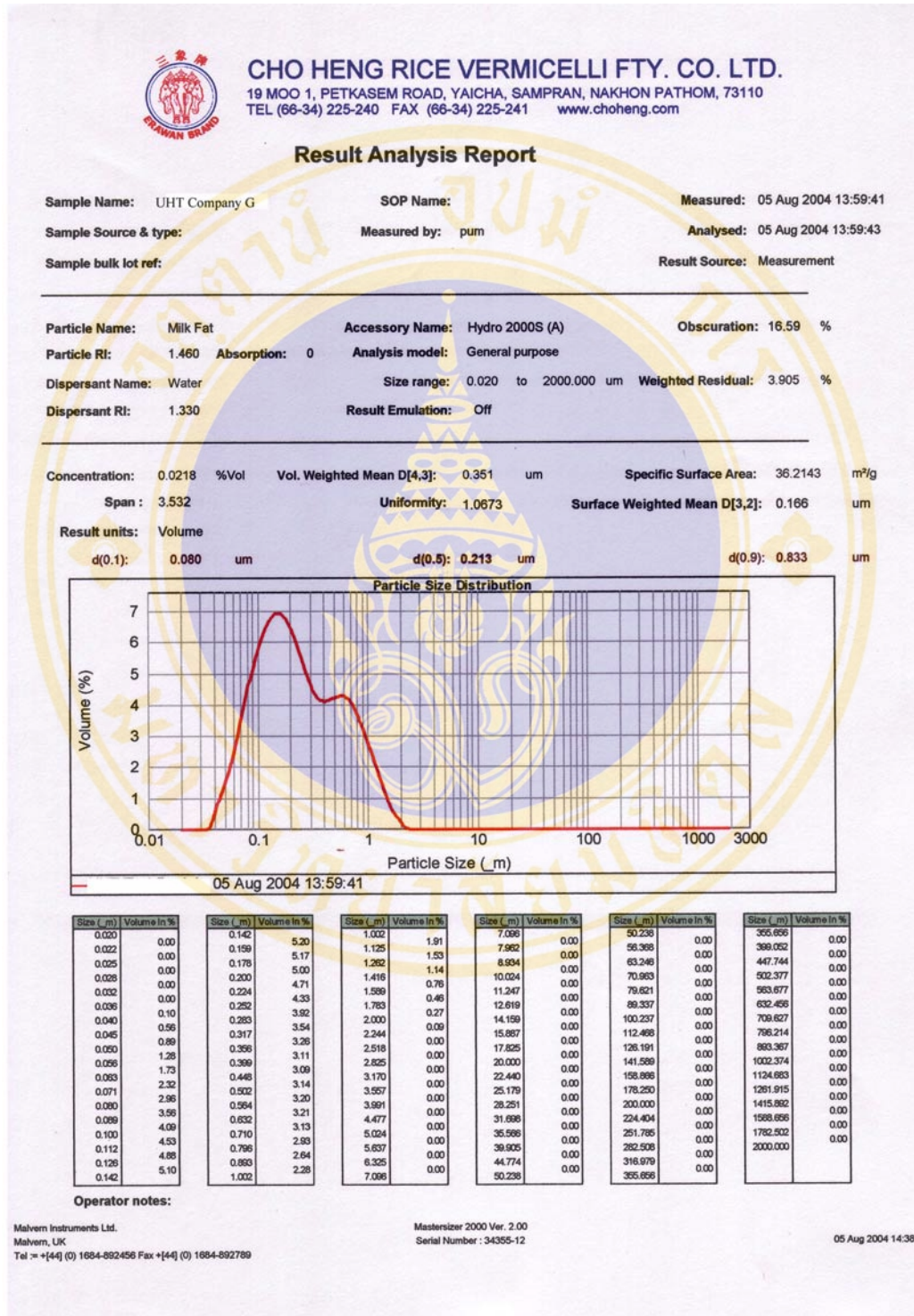


Figure 69 Particle size in UHT milk of company G

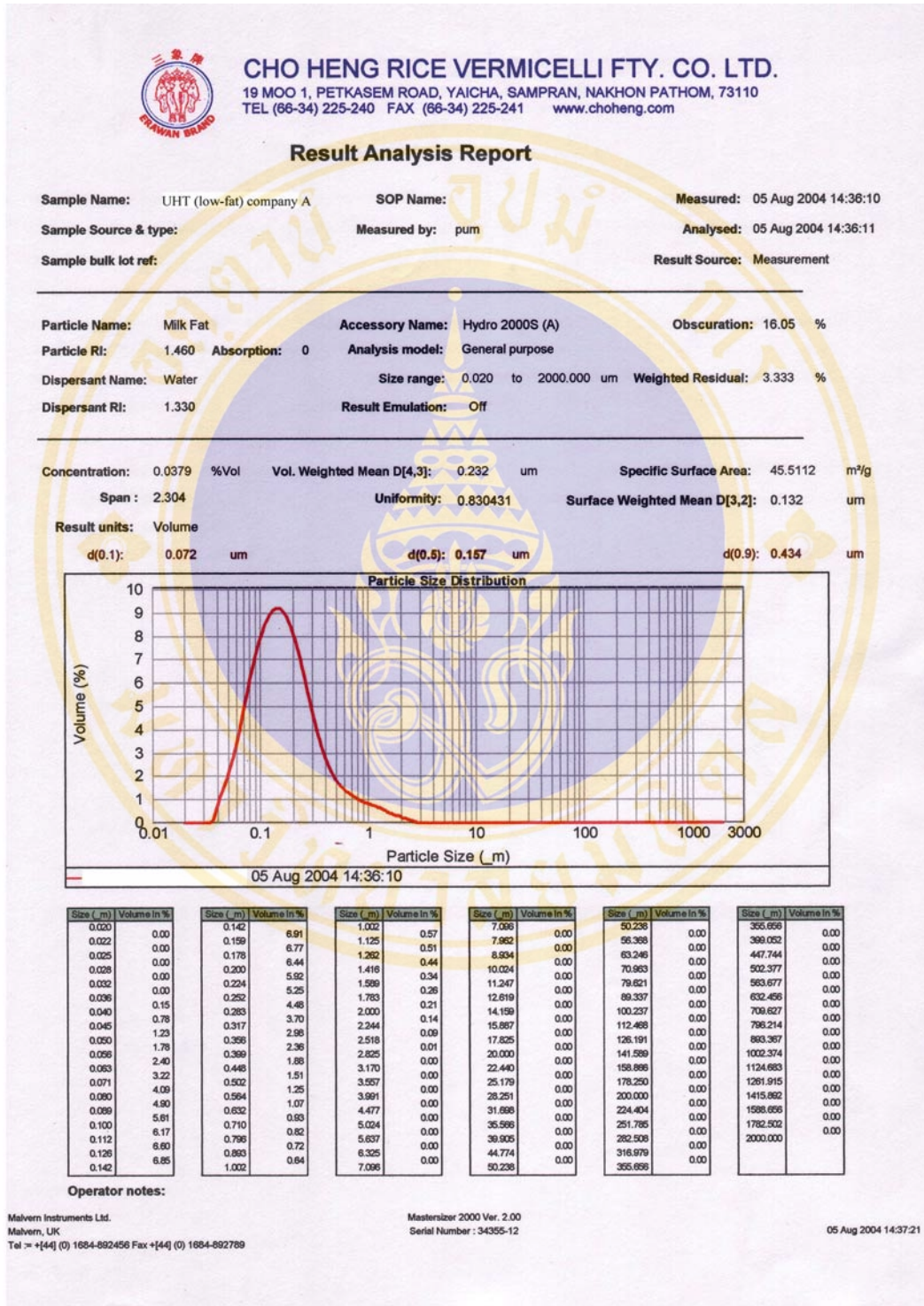


Figure 70 Particle size in UHT low-fat milk of company A

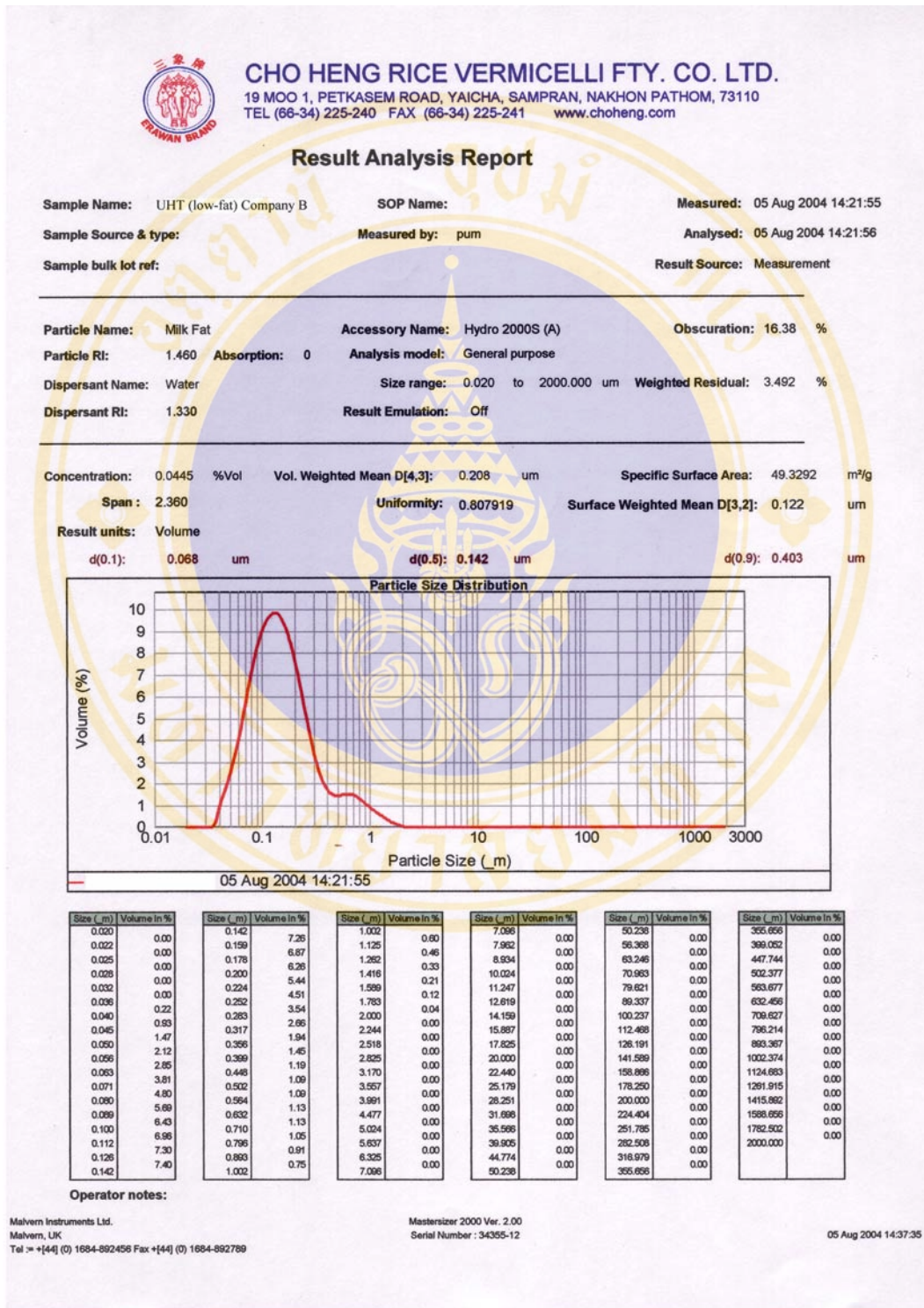


Figure 71 Particle size in UHT low-fat milk of company B

## Appendix B

(สำเนา)

ประกาศกระทรวงสาธารณสุข

(ฉบับที่ 265) พ.ศ. 2545

เรื่อง นมโค

โดยที่เป็นการสมควรปรับปรุงประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง นมโค

อาศัยอำนาจตามความในมาตรา 5 และมาตรา 6(1) (2) (4) (5) (6) (7) (9) และ (10) แห่งพระราชบัญญัติอาหาร พ.ศ.2522 อันเป็นพระราชบัญญัติที่มีบทบัญญัติบางประการเกี่ยวกับการจำกัดสิทธิและเสรีภาพของบุคคล ซึ่งมาตรา 29 ประกอบกับมาตรา 35 มาตรา 48 และมาตรา 50 ของรัฐธรรมนูญแห่งราชอาณาจักรไทยบัญญัติให้กระทำได้โดยอาศัยอำนาจตามบทบัญญัติแห่งกฎหมาย รัฐมนตรีว่าการกระทรวงสาธารณสุขออกประกาศไว้ ดังต่อไปนี้

ข้อ 1 ให้ยกเลิก

(1) ประกาศกระทรวงสาธารณสุข ฉบับที่ 26 (พ.ศ.2522) เรื่อง กำหนดนมโคเป็นอาหารควบคุมเฉพาะและกำหนดคุณภาพหรือมาตรฐานและวิธีการผลิต ลงวันที่ 13 กันยายน พ.ศ.2522

(2) ประกาศกระทรวงสาธารณสุข ฉบับที่ 149 (พ.ศ.2536) เรื่อง กำหนดนมโคเป็นอาหารควบคุมเฉพาะและกำหนดคุณภาพหรือมาตรฐานและวิธีการผลิต (ฉบับที่ 2) ลงวันที่ 11 ตุลาคม พ.ศ.2536

(3) ประกาศกระทรวงสาธารณสุข (ฉบับที่ 218) พ.ศ.2544 เรื่อง กำหนดนมโคเป็นอาหารควบคุมเฉพาะและกำหนดคุณภาพหรือมาตรฐานและวิธีการผลิต (ฉบับที่ 3) ลงวันที่ 8 มิถุนายน พ.ศ.2544

ข้อ 2 ให้นมโคเป็นอาหารควบคุมเฉพาะ

ข้อ 3 ในประกาศนี้ “นํ้านมดิบ” หมายความว่า นํ้านมที่รีดจากแม่โค

ข้อ 4 นมโค หมายความว่า ผลิตภัณฑ์ที่ได้จากการนำนํ้านมดิบมาผ่านกรรมวิธีการผลิตต่าง ๆ ให้มีลักษณะตามกระบวนการผลิตนั้น ๆ มี 5 ชนิด ได้แก่

(1) นํ้านมดิบที่ผ่านกรรมวิธีฆ่าเชื้อ

(2) นมผง

(3) นมข้น

(4) นมคั้นรูป

(5) นมแปลงไขมัน

ข้อ 5 นำนมดิบที่ผ่านกรรมวิธีฆ่าเชื้อ มี 3 ชนิด ได้แก่

(1) นำนมดิบชนิดเต็มมันเนยที่ผ่านกรรมวิธีฆ่าเชื้อ หมายถึง นำนมดิบที่มีได้แยกออกหรือเติมเข้าไปซึ่งวัตถุดิบใด เว้นแต่การปรับปริมาณมันเนยโดยการแยกหรือเติมมันเนย และต้องผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11

(2) นำนมดิบชนิดพร่องมันเนยที่ผ่านกรรมวิธีฆ่าเชื้อ หมายถึง นำนมดิบที่ได้แยกมันเนยออกบางส่วน และต้องผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11

(3) นำนมดิบชนิดขาดมันเนยที่ผ่านกรรมวิธีฆ่าเชื้อ หมายถึง นำนมดิบที่ได้แยกมันเนยออกเกือบทั้งหมด และต้องผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11

ข้อ 6 นมผง หมายความว่า นำนมดิบที่ผ่านกรรมวิธีฆ่าเชื้อที่ระเหยน้ำออกด้วยกรรมวิธีต่าง ๆ จนเป็นผง และอาจมีการเติมวัตถุดิบใดที่เป็นองค์ประกอบของนมอีกด้วยก็ได้ มี 3 ชนิด ได้แก่

(1) นมผงชนิดเต็มมันเนย

(2) นมผงชนิดพร่องมันเนย

(3) นมผงชนิดขาดมันเนย

ข้อ 7 นมข้น หมายความว่า นำนมดิบที่ระเหยเอาน้ำบางส่วนออกและอาจเติมน้ำตาลหรือวัตถุดิบใดที่เป็นองค์ประกอบของนมอีกด้วยก็ได้ มี 6 ชนิด ได้แก่

(1) นมข้นไม่หวานชนิดเต็มมันเนย

(2) นมข้นหวานชนิดเต็มมันเนย

(3) นมข้นไม่หวานชนิดพร่องมันเนย

(4) นมข้นหวานชนิดพร่องมันเนย

(5) นมข้นไม่หวานชนิดขาดมันเนย

(6) นมข้นหวานชนิดขาดมันเนย

ข้อ 8 นมคั้นรูป หมายความว่า ผลผลิตภัณฑ์ที่ได้จากการนำเอากองค์ประกอบของนมนมดิบมาผสมกันให้มีลักษณะเช่นเดียวกับนมโคตามข้อ 4(1) หรือ (3) และอาจเติมนำนมดิบหรือวัตถุดิบใดที่เป็นองค์ประกอบของนมอีกด้วยก็ได้ มี 9 ชนิด ได้แก่

(1) นมคั้นรูปชนิดเต็มมันเนย

- (2) นมกึ่งรูปชนิดพร้อมมันเนย
- (3) นมกึ่งรูปชนิดขาดมันเนย
- (4) นมชั้นกึ่งรูปไม่หวานชนิดเต็มมันเนย
- (5) นมชั้นกึ่งรูปหวานชนิดเต็มมันเนย
- (6) นมชั้นกึ่งรูปไม่หวานชนิดพร้อมมันเนย
- (7) นมชั้นกึ่งรูปหวานชนิดพร้อมมันเนย
- (8) นมชั้นกึ่งรูปไม่หวานชนิดขาดมันเนย
- (9) นมชั้นกึ่งรูปหวานชนิดขาดมันเนย

ข้อ 9 นมแปลงไขมัน หมายความว่า นมโคตามข้อ 4(1) (2) (3) หรือ (4) ที่ใช้ไขมันอื่นบางส่วนหรือทั้งหมดแทนมันเนยที่มีอยู่ มี 8 ชนิด ได้แก่

- (1) นมแปลงไขมันชนิดเต็มไขมัน
- (2) นมแปลงไขมันชนิดพร้อมไขมัน
- (3) นมผงแปลงไขมันชนิดเต็มไขมัน
- (4) นมผงแปลงไขมันชนิดพร้อมไขมัน
- (5) นมชั้นแปลงไขมันไม่หวานชนิดเต็มไขมัน
- (6) นมชั้นแปลงไขมันไม่หวานชนิดพร้อมไขมัน
- (7) นมชั้นแปลงไขมันหวานชนิดเต็มไขมัน
- (8) นมชั้นแปลงไขมันหวานชนิดพร้อมไขมัน

ข้อ 10 นมโคตามข้อ 6 ข้อ 7 ข้อ 8 หรือข้อ 9 อาจมีการเติมสารอาหารอื่น เพื่อเพิ่มชนิดและปริมาณสารอาหารนอกเหนือจากที่กำหนดในประกาศนี้ได้ โดยปฏิบัติตามหลักเกณฑ์วิธีการและเงื่อนไขที่กำหนดไว้ในประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง การเติมสารอาหารในผลิตภัณฑ์อาหาร

ข้อ 11 กรรมวิธีฆ่าเชื้อนมโคตามข้อ 4(1) ต้องเป็นกรรมวิธีฆ่าเชื้ออย่างใดอย่างหนึ่งดังต่อไปนี้

(1) พาสเจอร์ไรส์ หมายความว่า กรรมวิธีฆ่าเชื้อด้วยความร้อนที่อุณหภูมิไม่เกิน 100 องศาเซลเซียส โดยใช้อุณหภูมิและเวลาอย่างใดอย่างหนึ่ง ดังต่อไปนี้

(1.1) อุณหภูมิไม่ต่ำกว่า 63 องศาเซลเซียส และคงอยู่ที่อุณหภูมินี้ไม่น้อยกว่า 30 นาที แล้วทำให้เย็นลงทันทีที่อุณหภูมิ 5 องศาเซลเซียส หรือต่ำกว่า หรือ

(1.2) อุณหภูมิไม่ต่ำกว่า 72 องศาเซลเซียส และคงอยู่ที่อุณหภูมินี้ไม่น้อยกว่า 15 วินาทีแล้วทำให้เย็นลงทันทีที่อุณหภูมิ 5 องศาเซลเซียส หรือต่ำกว่า

(2) สเตอริไลส์ หมายความว่า กรรมวิธีฆ่าเชื้อนมโคตามข้อ 4(1) ที่บรรจุในภาชนะที่ปิดสนิทด้วยความร้อนที่อุณหภูมิไม่ต่ำกว่า 100 องศาเซลเซียส โดยใช้เวลาที่เหมาะสม ทั้งนี้จะต้องผ่านกรรมวิธีทำให้เป็นเนื้อเดียวกันด้วย

(3) ยู เอช ที หมายความว่า กรรมวิธีฆ่าเชื้อด้วยความร้อนที่อุณหภูมิไม่ต่ำกว่า 133 องศาเซลเซียส ไม่น้อยกว่า 1 วินาที แล้วบรรจุในภาชนะและในสภาวะที่ปราศจากเชื้อ ทั้งนี้จะต้องผ่านกรรมวิธีทำให้เป็นเนื้อเดียวกันด้วย

(4) กรรมวิธีอย่างอื่นที่มีมาตรฐานเทียบเท่ากรรมวิธีตาม (1) (2) หรือ (3) โดยได้รับความเห็นชอบจากคณะกรรมการอาหาร

ข้อ 12 นำนมดิบที่ผ่านกรรมวิธีฆ่าเชื้อ ต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) ต้องปราศจากเชื้อโรคอันอาจจะติดต่อกันได้ เช่น เชื้อที่ทำให้เกิดวัณโรค เชื้อที่ทำให้เกิดโรคแท้งติดต่อ เป็นต้น

(2) ไม่มีนํ้านมเน่าเหลืองเจือปน

(3) มีกลิ่นตามลักษณะเฉพาะของนํ้านมดิบที่ผ่านกรรมวิธีฆ่าเชื้อชนิดนั้น

(4) มีลักษณะเหลวเป็นเนื้อเดียวกัน

(5) ไม่มีสารที่อาจเป็นพิษ สารเป็นพิษจากจุลินทรีย์ และสารปนเปื้อน ในปริมาณที่อาจเป็นอันตรายต่อสุขภาพ เช่น สารตกค้างจากยาฆ่าแมลง สารปฏิชีวนะ แอฟลาทอกซิน เป็นต้น

(6) ไม่มีวัตถุกันเสีย

(7) ไม่มีวัตถุที่ให้ความหวานแทนน้ำตาล

(8) มีโปรตีนนมไม่น้อยกว่าร้อยละ 2.8 ของน้ำหนัก

(9) มีเนื้อมนมไม่รวมมันเนยและมันเนย ดังนี้

(9.1) เนื้อมนมไม่รวมมันเนยไม่น้อยกว่าร้อยละ 8.25 ของน้ำหนัก และมันเนยไม่น้อยกว่า ร้อยละ 3.2 ของน้ำหนัก สำหรับนํ้านมดิบชนิดเต็มมันเนยที่ผ่านกรรมวิธีฆ่าเชื้อ

(9.2) เนื้อมนมไม่รวมมันเนยไม่น้อยกว่าร้อยละ 8.5 ของน้ำหนัก และมันเนยมากกว่า ร้อยละ 0.1 ของน้ำหนัก แต่ไม่ถึงร้อยละ 3.2 ของน้ำหนัก สำหรับนํ้านมดิบชนิดพร่องมันเนยที่ผ่านกรรมวิธีฆ่าเชื้อ

(9.3) เนื้อมนมไม่รวมมันเนยไม่น้อยกว่าร้อยละ 8.8 ของน้ำหนัก และมันเนยไม่เกินร้อยละ 0.1 ของน้ำหนัก สำหรับนํ้านมดิบชนิดขาดมันเนยที่ผ่านกรรมวิธีฆ่าเชื้อ

(10) ไม่มีจุลินทรีย์ที่ทำให้เกิดโรค

(11) ตรวจไม่พบแบคทีเรียชนิด อี.โคไล (*Escherichia coli*) ในน้ำนมดิบที่ผ่านกรรมวิธีฆ่าเชื้อ 0.1 มิลลิลิตร

(12) ตรวจพบแบคทีเรียในน้ำนมดิบที่ผ่านกรรมวิธีพาสเจอร์ไรส์ 1 มิลลิลิตร ได้ไม่เกิน 10,000 หน แหล่งผลิตและไม่เกิน 50,000 ตลอดระยะเวลาเมื่อออกจากแหล่งผลิตจนถึงวันหมดอายุ การบริโภคที่ระบุบนฉลาก

(13) ตรวจพบแบคทีเรียชนิดโคลิฟอร์มได้ไม่เกิน 100 ในน้ำนมดิบที่ผ่านกรรมวิธีพาสเจอร์ไรส์ 1 มิลลิลิตร หน แหล่งผลิต

(14) ตรวจไม่พบแบคทีเรียในน้ำนมดิบที่ผ่านกรรมวิธีสเตอริไลส์และน้ำนมดิบที่ผ่านกรรมวิธียู เอช ที 0.1 มิลลิลิตร

ข้อ 13 น้ำนมดิบที่ผ่านกรรมวิธีพาสเจอร์ไรส์ต้องเก็บรักษาไว้ที่อุณหภูมิไม่เกิน 8 องศาเซลเซียสตลอดระยะเวลาหลังบรรจุจนถึงผู้บริโภค และระยะเวลาการบริโภคต้องไม่เกิน 10 วัน นับจากวันที่บรรจุในภาชนะบรรจุพร้อมจำหน่าย

กรณีที่จะแสดงระยะเวลาการบริโภคเกินกว่าที่กำหนดตามวรรคหนึ่ง ต้องมีมาตรการในการควบคุมคุณภาพหรือมาตรฐานผลิตภัณฑ์ตลอดระยะเวลาตั้งแต่หลังการบรรจุถึงการจำหน่ายถึงผู้บริโภคเป็นไปตามหลักเกณฑ์ที่ได้รับความเห็นชอบจากคณะกรรมการอาหาร

ข้อ 14 น้ำนมดิบที่ผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11(2) หรือ (3) ต้องเก็บรักษาไว้ที่อุณหภูมิปกติในระยะเวลาไม่น้อยกว่า 5 วัน นับแต่วันที่บรรจุในภาชนะก่อนออกจำหน่าย เพื่อตรวจสอบว่ายังคงมีคุณภาพหรือมาตรฐานตามที่กำหนด และไม่เปลี่ยนแปลงไปจากลักษณะเดิมที่ทำขึ้น

ข้อ 15 นมผงต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) มีกลิ่นตามลักษณะเฉพาะของนมผงชนิดนั้น

(2) มีลักษณะเป็นผงไม่เกาะเป็นก้อน

(3) มีความชื้นไม่เกินร้อยละ 5 ของน้ำหนัก

(4) ไม่มีสารที่อาจเป็นพิษ สารเป็นพิษจากจุลินทรีย์ และสารปนเปื้อน ในปริมาณที่อาจเป็นอันตรายต่อสุขภาพ เช่น สารตกค้างจากยาฆ่าแมลง สารปฏิชีวนะ แอฟลาทอกซิน เป็นต้น

(5) ไม่มีวัตถุกันเสีย

(6) ไม่มีวัตถุที่ทำให้ความหวานแทนน้ำตาล

(7) มีโปรตีนนมในเนื้อนมไม่รวมมันเนยไม่น้อยกว่าร้อยละ 34 ของน้ำหนัก

(8) มีมันเนยดังนี้

- (8.1) ไม่น้อยกว่าร้อยละ 26 ของน้ำหนัก สำหรับนมผงชนิดเต็มมันเนย
- (8.2) มากกว่าร้อยละ 1.5 ของน้ำหนัก แต่ไม่ถึงร้อยละ 26 ของน้ำหนัก สำหรับนมผงชนิดพร่องมันเนย
- (8.3) ไม่เกินร้อยละ 1.5 ของน้ำหนัก สำหรับนมผงชนิดขาดมันเนย
- (9) ไม่มีจุลินทรีย์ที่ทำให้เกิดโรค
- (10) ตรวจไม่พบแบคทีเรียชนิด อี. โคไล (*Escherichia coli*) ในนมผง 0.1 กรัม
- (11) ตรวจพบแบคทีเรียได้ไม่เกิน 50,000 ในนมผง 1 กรัม
- ข้อ 16 นมชั้นต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้
- (1) มีกลิ่นตามลักษณะเฉพาะของนมชั้นชนิดนั้น
  - (2) มีลักษณะเป็นเนื้อเดียวกัน ไม่เป็นก้อน
  - (3) ไม่มีวัตถุกันเสีย
  - (4) ไม่มีวัตถุที่ทำให้ความหวานแทนน้ำตาล
  - (5) ไม่มีสารที่อาจเป็นพิษ สารเป็นพิษจากจุลินทรีย์ และสารปนเปื้อน ในปริมาณที่อาจเป็นอันตรายต่อสุขภาพ เช่น สารตกค้างจากยาฆ่าแมลง สารปฏิชีวนะ แอฟลาทอกซิน เป็นต้น
  - (6) มีโปรตีนนมในเนื้อมนมรวมมันเนยไม่น้อยกว่าร้อยละ 34 ของน้ำหนัก
  - (7) มีเนื้อมนมและมันเนย ดังนี้
    - (7.1) เนื้อมนมไม่น้อยกว่าร้อยละ 25 ของน้ำหนัก และมันเนยไม่น้อยกว่าร้อยละ 7.5 ของน้ำหนัก สำหรับนมชั้นไม่หวานชนิดเต็มมันเนย
    - (7.2) เนื้อมนมไม่น้อยกว่าร้อยละ 28 ของน้ำหนัก และมันเนยไม่น้อยกว่าร้อยละ 8 ของน้ำหนัก สำหรับนมชั้นหวานชนิดเต็มมันเนย
    - (7.3) เนื้อมนมไม่น้อยกว่าร้อยละ 20 ของน้ำหนัก และมันเนยมากกว่าร้อยละ 1 ของน้ำหนัก แต่ไม่ถึงร้อยละ 7.5 ของน้ำหนัก สำหรับนมชั้นไม่หวานชนิดพร่องมันเนย
    - (7.4) เนื้อมนมไม่น้อยกว่าร้อยละ 24 ของน้ำหนัก และมันเนยมากกว่าร้อยละ 1 ของน้ำหนัก แต่ไม่ถึงร้อยละ 8 ของน้ำหนัก สำหรับนมชั้นหวานชนิดพร่องมันเนย
    - (7.5) เนื้อมนมไม่น้อยกว่าร้อยละ 20 ของน้ำหนัก และมันเนยไม่เกินร้อยละ 1 ของน้ำหนัก สำหรับนมชั้นไม่หวานชนิดขาดมันเนย
    - (7.6) เนื้อมนมไม่น้อยกว่าร้อยละ 24 ของน้ำหนัก และมันเนยไม่เกินร้อยละ 1 ของน้ำหนัก สำหรับนมชั้นหวานชนิดขาดมันเนย
  - (8) มีวิตามินเอไม่น้อยกว่า 330 ไมโครกรัมเรตินอล ต่อนมชั้นหวาน 100 กรัม

(9) ไม่มีจุลินทรีย์ที่ทำให้เกิดโรค

(10) ตรวจพบยีสต์และเชื้อรารวมกันได้ไม่เกิน 10 ในนมข้นหวาน 1 กรัม

(11) ตรวจไม่พบแบคทีเรียชนิดโคลิฟอร์มในนมข้นหวาน 0.1 กรัม

(12) ตรวจพบแบคทีเรียได้ไม่เกิน 10,000 ในนมข้นหวาน 1 กรัม

(13) ตรวจไม่พบแบคทีเรียในนมข้นไม่หวาน 0.1 มิลลิลิตร

ข้อ 17 นมคั้นรูป ต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) นมคั้นรูปชนิดเต็มมันเนยมีคุณภาพหรือมาตรฐานเช่นเดียวกับน้ำมันดิบชนิดเต็มมันเนยที่ผ่านกรรมวิธีฆ่าเชื้อ

(2) นมคั้นรูปชนิดพร่องมันเนยมีคุณภาพหรือมาตรฐานเช่นเดียวกับน้ำมันดิบชนิดพร่องมันเนยที่ผ่านกรรมวิธีฆ่าเชื้อ

(3) นมคั้นรูปชนิดขาดมันเนยมีคุณภาพหรือมาตรฐานเช่นเดียวกับน้ำมันดิบชนิดขาดมันเนยที่ผ่านกรรมวิธีฆ่าเชื้อ

(4) นมข้นคั้นรูปไม่หวานชนิดเต็มมันเนยมีคุณภาพหรือมาตรฐานเช่นเดียวกับนมข้นไม่หวานชนิดเต็มมันเนย

(5) นมข้นคั้นรูปหวานชนิดเต็มมันเนยมีคุณภาพหรือมาตรฐานเช่นเดียวกับนมข้นหวานชนิดเต็มมันเนย

(6) นมข้นคั้นรูปไม่หวานชนิดพร่องมันเนยมีคุณภาพหรือมาตรฐานเช่นเดียวกับนมข้นไม่หวานชนิดพร่องมันเนย

(7) นมข้นคั้นรูปหวานชนิดพร่องมันเนยมีคุณภาพหรือมาตรฐานเช่นเดียวกับนมข้นหวานชนิดพร่องมันเนย

(8) นมข้นคั้นรูปไม่หวานชนิดขาดมันเนยมีคุณภาพหรือมาตรฐานเช่นเดียวกับนมข้นไม่หวานชนิดขาดมันเนย

(9) นมข้นคั้นรูปหวานชนิดขาดมันเนยมีคุณภาพหรือมาตรฐานเช่นเดียวกับนมข้นหวานชนิดขาดมันเนย

ข้อ 18 นมคั้นรูปตามข้อ 8(1)(2) และ (3) ต้องผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11 และต้องปฏิบัติดังต่อไปนี้

(1) กรณีที่ผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11(1) ต้องปฏิบัติตามข้อ 13

(2) กรณีที่ผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11(2) หรือ (3) ต้องปฏิบัติตามข้อ 14

ข้อ 19 นมแปลงไขมัน ต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) นมแปลงไขมันชนิดเต็มไขมันต้องมีเนื้อมันไม่รวมไขมันไม่น้อยกว่าร้อยละ 8.25 ของน้ำหนักและมีไขมันทั้งหมดไม่น้อยกว่าร้อยละ 3.2 ของน้ำหนัก และต้องมีคุณภาพหรือมาตรฐานตามข้อ 12(1)(2)(3)(4)(5)(6)(7)(8)(10)(11)(12)(13) และ (14)

(2) นมแปลงไขมันชนิดพร่องไขมันต้องมีเนื้อมันไม่รวมไขมันไม่น้อยกว่าร้อยละ 8.5 ของน้ำหนักและมีไขมันทั้งหมดมากกว่าร้อยละ 0.1 ของน้ำหนัก แต่ไม่ถึงร้อยละ 3.2 ของน้ำหนัก และต้องมีคุณภาพหรือมาตรฐานตามข้อ 12(1)(2)(3)(4)(5)(6)(7)(8)(10)(11)(12)(13) และ (14)

(3) นมผงแปลงไขมันชนิดเต็มไขมัน ต้องมีไขมันทั้งหมดไม่น้อยกว่าร้อยละ 26 ของน้ำหนักและต้องมีคุณภาพหรือมาตรฐานตามข้อ 15(1) (2) (3) (4) (5) (6) (7) (9) (10) และ (11)

(4) นมผงแปลงไขมันชนิดพร่องไขมัน ต้องมีไขมันทั้งหมดมากกว่าร้อยละ 1.5 ของน้ำหนักแต่ไม่ถึงร้อยละ 26 ของน้ำหนัก และต้องมีคุณภาพหรือมาตรฐานตามข้อ 15(1) (2) (3) (4) (5) (6) (7) (9) (10) และ (11)

(5) นมข้นแปลงไขมันไม่หวานชนิดเต็มไขมัน ต้องมีเนื้อมันไม่รวมไขมันไม่น้อยกว่าร้อยละ 17.5 ของน้ำหนัก และมีไขมันทั้งหมดไม่น้อยกว่าร้อยละ 6 ของน้ำหนักและต้องมีคุณภาพหรือมาตรฐานตามข้อ 16 (1) (2) (3) (4) (5) (6) (9) และ (13)

(6) นมข้นแปลงไขมันไม่หวานชนิดพร่องไขมัน ต้องมีเนื้อมันไม่น้อยกว่าร้อยละ 20 ของน้ำหนักและมีไขมันทั้งหมดมากกว่าร้อยละ 1 ของน้ำหนัก แต่ไม่ถึงร้อยละ 6 ของน้ำหนัก และต้องมีคุณภาพหรือมาตรฐานตามข้อ 16 (1) (2) (3) (4) (5) (6) (9) และ (13)

(7) นมข้นแปลงไขมันหวานชนิดเต็มไขมัน ต้องมีเนื้อมันไม่รวมไขมันไม่น้อยกว่าร้อยละ 20 ของน้ำหนัก และมีไขมันทั้งหมดไม่น้อยกว่าร้อยละ 7 ของน้ำหนัก และต้องมีคุณภาพหรือมาตรฐานตามข้อ 16 (1) (2) (3) (4) (5) (6) (8) (9) (10) (11) และ (12)

(8) นมข้นแปลงไขมันหวานชนิดพร่องไขมันต้องมีเนื้อมันไม่น้อยกว่าร้อยละ 24 ของน้ำหนักและมีไขมันทั้งหมดมากกว่าร้อยละ 1 ของน้ำหนักแต่ไม่ถึงร้อยละ 7 ของน้ำหนัก และต้องมีคุณภาพหรือมาตรฐานตามข้อ 16(1) (2) (3) (4) (5) (6) (8) (9) (10) (11) และ (12)

ข้อ 20 นมแปลงไขมันตามข้อ 9(1) และ (2) ต้องผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11 และต้องปฏิบัติตามดังต่อไปนี้

(1) กรณีที่ผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11(1) ต้องปฏิบัติตามข้อ 13

(2) กรณีที่ผ่านกรรมวิธีฆ่าเชื้อตามข้อ 11(2) หรือ (3) ต้องปฏิบัติตามข้อ 14

ข้อ 21 การผลิตนมโคถ้าจำเป็นต้องใช้วัตถุเจือปนอาหารนอกจากวัตถุกันเสีย ให้ใช้ได้ตามหลักเกณฑ์ที่กำหนดไว้ในบัญชีแนบท้ายประกาศนี้

ข้อ 22 ผู้ผลิตหรือผู้นำเข้านมโคเพื่อจำหน่าย ต้องปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง วิธีการผลิต เครื่องมือเครื่องใช้ในการผลิต และการเก็บรักษาอาหาร

ข้อ 23 การใช้ภาชนะบรรจุนมโค ให้ปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง ภาชนะบรรจุ

ข้อ 24 การแสดงฉลากของนมโค ให้ปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง ฉลาก เว้นแต่การใช้ชื่อนมโคและการแสดงข้อความสำหรับนมโคบางชนิด ให้ปฏิบัติดังต่อไปนี้

(1) การใช้ชื่ออาหารของนมโค ได้แก่

(1.1) นำนมดิบที่ผ่านกรรมวิธีฆ่าเชื้อตามข้อ 5 ให้ใช้ชื่อดังนี้

(1.1.1) “นม.....” (ความที่เว้นไว้ให้ระบุกรรมวิธีฆ่าเชื้อตามข้อ

11) สำหรับชนิดเต็มมันเนย

(1.1.2) “นม.....พร้อมมันเนย” หรือ “นมพร้อมมันเนย.....”

(ความที่เว้นไว้ให้ระบุกรรมวิธีฆ่าเชื้อตามข้อ 11) สำหรับชนิดพร้อมมันเนย

(1.1.3) “นม.....ขาดมันเนย” หรือ “นมขาดมันเนย.....” (ความที่

เว้นไว้ให้ระบุกรรมวิธีฆ่าเชื้อตามข้อ 11) สำหรับชนิดขาดมันเนย

(1.2) นมผงตามข้อ 6 ให้ใช้ชื่อดังนี้

(1.2.1) “นมผง” สำหรับนมผงชนิดเต็มมันเนย

(1.2.2) “นมผงพร้อมมันเนย” สำหรับนมผงชนิดพร้อมมันเนย

(1.2.3) “นมผงขาดมันเนย” สำหรับนมผงชนิดขาดมันเนย

(1.3) นมข้น ตามข้อ 7 ให้ใช้ชื่อดังนี้

(1.3.1) “นมข้นไม่หวาน” สำหรับนมข้นไม่หวานชนิดเต็มมันเนย

(1.3.2) “นมข้นหวาน” สำหรับนมข้นหวานชนิดเต็มมันเนย

(1.3.3) “นมข้นไม่หวานพร้อมมันเนย” สำหรับนมข้นไม่หวานชนิด

พร้อมมันเนย

(1.3.4) “นมข้นหวานพร้อมมันเนย” สำหรับนมข้นหวานชนิด

พร้อมมันเนย

(1.3.5) “นมข้นไม่หวานขาดมันเนย” สำหรับนมข้นไม่หวานชนิด

ขาดมันเนย

(1.3.6) “นมข้นหวานขาดมันเนย” สำหรับนมข้นหวานชนิดขาด

มันเนย

(1.4) นมคีนรูปตามข้อ 8 ให้ใช้ชื่อดังนี้

(1.4.1) “นมคีนรูป.....” (ความที่เว้นไว้ให้ระบุกรรมวิธีฆ่าเชื้อตามข้อ 11) สำหรับนมคีนรูปชนิดเต็มมันเนย

(1.4.2) “นมคีนรูปพร่องมันเนย.....” หรือ “นมคีนรูป .....พร่องมันเนย”(ความที่เว้นไว้ให้ระบุกรรมวิธีฆ่าเชื้อตามข้อ11) สำหรับนมคีนรูปชนิดพร่องมันเนย

(1.4.3) “นมคีนรูปขาดมันเนย.....” หรือ “นมคีนรูป .....ขาดมันเนย” (ความที่เว้นไว้ให้ระบุกรรมวิธีฆ่าเชื้อตามข้อ 11) สำหรับนมคีนรูปชนิดขาดมันเนย

(1.4.4) “นมข้นคีนรูปไม่หวาน” สำหรับนมข้นคีนรูปไม่หวานชนิดเต็มมันเนย

1.4.5) “นมข้นคีนรูปหวาน” สำหรับนมข้นคีนรูปหวานชนิดเต็มมันเนย

(1.4.6) “นมข้นคีนรูปไม่หวานพร่องมันเนย” สำหรับนมข้นคีนรูปไม่หวานชนิดพร่องมันเนย

(1.4.7) “นมข้นคีนรูปหวานพร่องมันเนย” สำหรับนมข้นคีนรูปหวานชนิดพร่องมันเนย

(1.4.8) “นมข้นคีนรูปไม่หวานขาดมันเนย” สำหรับนมข้นคีนรูปไม่หวานชนิดขาดมันเนย

(1.4.9) “นมข้นคีนรูปหวานขาดมันเนย” สำหรับนมข้นคีนรูปหวานชนิดขาดมันเนย

(1.5) นมแปลงไขมันตามข้อ 9 ให้ใช้ชื่อดังนี้

(1.5.1) “นมแปลงไขมัน.....” (ความที่เว้นไว้ให้ระบุกรรมวิธีฆ่าเชื้อตามข้อ11) สำหรับนมแปลงไขมันชนิดเต็มไขมัน

(1.5.2) “นมแปลงไขมันชนิดพร่องไขมัน .....” หรือ “นมแปลงไขมัน .....ชนิดพร่องไขมัน” (ความที่เว้นไว้ให้ระบุกรรมวิธีฆ่าเชื้อตามข้อ 11) สำหรับนมแปลงไขมันชนิดพร่องไขมัน

(1.5.3) “นมผงดแปลงไขมัน” สำหรับนมผงดแปลงไขมันชนิดเต็มไขมัน

(1.5.4) “นมผงแปลงไขมันชนิดพร่องไขมัน” สำหรับนมผงแปลงไขมันชนิดพร่องไขมัน

(1.5.5) “นมข้นแปลงไขมันไม่หวาน” สำหรับนมข้นแปลงไขมันไม่หวานชนิดเต็มไขมัน

(1.5.6) “นมข้นแปลงไขมันไม่หวานชนิดพร่องไขมัน” สำหรับนมข้นแปลงไขมันไม่หวานชนิดพร่องไขมัน

(1.5.7) “นมข้นแปลงไขมันหวาน” สำหรับนมข้นแปลงไขมันหวานชนิดเต็มไขมัน

(1.5.8) “นมข้นแปลงไขมันหวานชนิดพร่องไขมัน” สำหรับนมข้นแปลงไขมันหวานชนิดพร่องไขมัน

การใช้ชื่ออาหารของนมโคอาจใช้ชื่อทางการค้าได้ แต่ต้องมีข้อความตาม (1) แล้วแต่ชนิดของนมโคกำกับชื่ออาหารด้วย โดยจะแสดงอยู่ในบรรทัดเดียวกับชื่อทางการค้าก็ได้ และจะมีขนาดตัวอักษรต่างกับชื่อทางการค้าก็ได้ แต่ต้องสามารถอ่านได้ชัดเจน

(2) การแสดงข้อความสำหรับนมโคบางชนิด ดังนี้

(2.1) ข้อความว่า “อย่าใช้เลี้ยงทารก” ด้วยตัวอักษรเส้นทึบสีแดง ขนาดความสูงไม่น้อยกว่า 5 มิลลิเมตร ในกรอบสี่เหลี่ยมพื้นขาว สีของกรอบตัดกับสีพื้นของฉลาก สำหรับนมโคตามข้อ 6(2) และ (3) ข้อ 7(3) และ (5) ข้อ 8(6) และ (8) และข้อ 9(1)(2)(3)(4)(5) และ (6)

(2.2) ข้อความว่า “อย่าใช้เลี้ยงทารกอายุต่ำกว่า 1 ปี” ด้วยตัวอักษรเส้นทึบสีแดง ขนาดความสูงไม่น้อยกว่า 5 มิลลิเมตร ในกรอบสี่เหลี่ยมพื้นขาว สีของกรอบตัดกับสีของพื้นฉลาก สำหรับนมโคตามข้อ 7(2) (4) และ (6) ข้อ 8(5) (7) และ (9) และข้อ 9(7) และ (8)

ข้อ 25 ผู้ที่ได้รับใบสำคัญการขึ้นทะเบียนตำรับอาหาร ซึ่งออกให้ตามประกาศกระทรวงสาธารณสุข ฉบับที่ 26 (พ.ศ.2522) เรื่อง กำหนดนมโคเป็นอาหารควบคุมเฉพาะและกำหนดคุณภาพหรือมาตรฐานและวิธีการผลิต ลงวันที่ 13 กันยายน พ.ศ.2522 ซึ่งแก้ไขเพิ่มเติมโดยประกาศกระทรวงสาธารณสุขฉบับที่ 149 (พ.ศ.2536) เรื่อง กำหนดนมโคเป็นอาหารควบคุมเฉพาะและกำหนดคุณภาพหรือมาตรฐานและวิธีการผลิต (ฉบับที่ 2) ลงวันที่ 11 ตุลาคม พ.ศ.2536 และประกาศกระทรวงสาธารณสุข (ฉบับที่ 218) พ.ศ.2544 เรื่อง กำหนดนมโคเป็นอาหารควบคุมเฉพาะและกำหนดคุณภาพหรือมาตรฐานและวิธีการผลิต (ฉบับที่ 3) ลงวันที่ 8 มิถุนายน พ.ศ.2544 และผู้ที่ได้รับใบสำคัญการใช้ฉลากอาหารซึ่งออกให้ตามประกาศกระทรวงสาธารณสุข ฉบับที่ 68 (พ.ศ. 2525) เรื่อง ฉลาก ลงวันที่ 29 เมษายน พ.ศ.2525 ซึ่งแก้ไขเพิ่มเติมโดยประกาศกระทรวง

สาธารณสุข ฉบับที่ 95 (พ.ศ.2528) เรื่อง ฉลาก (ฉบับที่ 2) ลงวันที่ 30 กันยายน พ.ศ.2528 หรือประกาศกระทรวงสาธารณสุข (ฉบับที่ 194) พ.ศ.2543 เรื่อง ฉลาก ลงวันที่ 19 กันยายน พ.ศ.2543 ซึ่งแก้ไขเพิ่มเติมโดยประกาศกระทรวงสาธารณสุข (ฉบับที่ 252) พ.ศ.2545 เรื่อง ฉลาก (ฉบับที่ 2) ลงวันที่ 30 พฤษภาคม พ.ศ.2545 อยู่ก่อนวันที่ประกาศนี้ใช้บังคับให้ปฏิบัติ ดังนี้

(1) ยื่นคำขอแก้ไขรายละเอียดให้ถูกต้องตามประกาศนี้ ภายในหนึ่งร้อยแปดสิบวันนับแต่วันที่ประกาศนี้ใช้บังคับ และเมื่อได้ยื่นคำขอดังกล่าวแล้วให้ใช้ฉลากเดิมที่เหลืออยู่ต่อไปได้ ดังนี้

(1.1) ฉลากที่ไม่แสดงเลขสารบบอาหาร ให้ใช้ได้ไม่เกินวันที่ 23 กรกฎาคม พ.ศ.2546

(1.2) ฉลากที่แสดงเลขสารบบอาหาร ให้ใช้ได้ไม่เกินหนึ่งปีนับแต่วันที่ประกาศนี้ใช้บังคับ

(2) ดำเนินการให้เป็นไปตามข้อ 22 ภายในวันที่ 23 กรกฎาคม พ.ศ.2546

ข้อ 26 ประกาศนี้ ให้ใช้บังคับตั้งแต่วันถัดจากวันประกาศในราชกิจจานุเบกษาเป็นต้นไป

ประกาศ ณ วันที่ 19 ธันวาคม พ.ศ. 2545

ลงชื่อ สุดารัตน์ เกตุราพันธ์

(นางสุดารัตน์ เกตุราพันธ์)

รัฐมนตรีว่าการกระทรวงสาธารณสุข

(คัดจากราชกิจจานุเบกษาฉบับประกาศทั่วไป เล่ม 120 ตอนพิเศษ 4 ง. ลงวันที่ 10 มกราคม พ.ศ. 2546)

บัญชีแนบท้ายประกาศกระทรวงสาธารณสุข (ฉบับที่ 265) พ.ศ.2545 เรื่อง นมโค

1. หลักเกณฑ์การใช้วัตถุเจือปนอาหารในนมผงและนมผงแปลงไขมัน

| อันดับ | วัตถุประสงค์   | ชื่อวัตถุเจือปนอาหาร  | ปริมาณสูงสุดที่ให้ได้<br>(มิลลิกรัมต่อ 1 กิโลกรัม)              |
|--------|--|---|---|
| 1.     | สตาบิไลเซอร์<br>(Stabilizers)                            | โซเดียมซิเตรต (Sodium citrates)<br>โพแทสเซียมซิเตรต (Potassium citrates)  | 5,000 ใช้อย่างเดียว หรือใช้<br>ร่วมกันคำนวณในสภาพ<br>ปราศจากน้ำ |
| 2.     | วัตถุทำให้คงรูป<br>(Firming agents)                      | โพแทสเซียมคลอไรด์ (Potassium chloride)<br>แคลเซียมคลอไรด์ (Calcium chloride)  | ปริมาณที่เหมาะสมตามความ<br>จำเป็นในการผลิต                      |
| 3.     | สารปรับความเป็น<br>กรด - ด่าง<br>(Acidity<br>Regulators) | โซเดียมฟอสเฟต<br>(Sodium phosphates)<br>โพแทสเซียมฟอสเฟต<br>(Potassium phosphates)<br>ไดฟอสเฟต (Diphosphates)<br>ไตรฟอสเฟต (Triphosphates)<br>โพลีฟอสเฟต (Polyphosphates)<br>โซเดียม คาร์บอเนต (Sodium carbonates)<br>โพแทสเซียมคาร์บอเนต<br>(Potassium carbonates) | 5,000 ใช้อย่างเดียว<br>หรือใช้ร่วมกัน<br>คำนวณในสภาพปราศจากน้ำ  |

|    |   |  |  |
|----|---|--|--|
| 4. | อิมัลซิไฟเออร์<br>(Emulsifiers)                         | เลซิทิน (Lecithins or phospholipids from natural sources)  | ปริมาณที่เหมาะสมตามความ<br>จำเป็นในการผลิต |
|    |   | โมนอและไดกลีเซอไรด์ (Mono - and diglycerides of fatty acide)   | 2,500                                      |
| 5. | วัตถุกันการรวมตัว<br>เป็นก้อน (Anti -<br>caking Agents) | แคลเซียมคาร์บอเนต (Calcium carbonate)<br><br>ไตรแคลเซียม ออโรฟอสเฟต (Tricalcium orthophosphate)<br><br>ไตรแมกนีเซียม ออโร ฟอสเฟต (Trimagnesium orthophosphate)<br><br>แมกนีเซียมคาร์บอเนต (Magnesium carbonate)<br><br>แมกนีเซียม ออกไซด์ (Magnesium oxide)<br><br>ซิลิคอน ไดออกไซด์ (Silicon dioxide, amorphous)<br><br>แคลเซียม ซิลิเกต (Calcium silicate)<br><br>แมกนีเซียมซิลิเกต (Magnesium silicate)<br><br>โซเดียม อะลูมิโนซิลิเกต (Sodium aluminosilicate) | 10,000 ใช้อย่างเดียว<br>หรือใช้ร่วมกัน     |

|    |                               |  |                            |
|----|-------------------------------|--|----------------------------|
|    |                               | แคลเซียม อะลูมิเนียม ซิลิเกต<br>(Calcium aluminium silicate) |                            |
|    |                               | อะลูมิเนียม ซิลิเกต (Aluminium silicate)                     |                            |
|    |                               | โพลีไดเมทิลซิลอกเซน<br>(Polydimethylsiloxane)                | 10                         |
| 6. | วัตถุกันหืน<br>(Antioxidants) | กรดแอสคอร์บิก (L - Ascorbic acid)                            | 500 จำนวนเป็นกรดแอสคอร์บิก |
|    |                               | โซเดียมแอสคอร์เบต (Sodium ascorbate)                         |                            |
|    |                               | แอสคอร์บิลปาล์มิเตต (Ascorbyl palmitate)                     |                            |
|    |                               | บิวทิลเตตไฮดรอกซีอะนิโซล<br>(Butylated hydroxyanisole BHA)   | 100                        |

2. หลักเกณฑ์การใช้วัตถุเจือปนอาหารในนมข้น นมข้นจืดรูปและนมข้นแปลงไขมัน

| อันดับ | วัตถุประสงค์   | ชื่อวัตถุเจือปนอาหาร  | ปริมาณสูงสุดที่ให้ได้<br>(มิลลิกรัมต่อ 1 กิโลกรัม)                        |
|--------|--|---|---|
| 1.     | สเตบิไลเซอร์<br>(Stabilizers)                            | โซเดียมซิเตรต (Sodium citrates)<br>โพแทสเซียมซิเตรต (Potassium citrates)<br>แคลเซียมซิเตรต (Calcium citrates)   | 2,000 ใช้อย่างเดียว หรือ 3,000<br>ใช้ร่วมกันคำนวณในสภาพที่<br>ปราศจากน้ำ  |
| 2.     | วัตถุทำให้คงรูป<br>(Firming agents)                      | โพแทสเซียม คลอไรด์<br>(Potassiumchloride)<br>แคลเซียม คลอไรด์ (Calcium chloride)  | 2,000 ใช้อย่างเดียว หรือ 3,000<br>ใช้ร่วมกัน คำนวณในสภาพที่<br>ปราศจากน้ำ |
| 3.     | สารปรับความเป็น<br>กรด - ด่าง<br>(Acidity<br>Regulators) | แคลเซียม คาร์บอเนต<br>(Calciumcarbonates)<br>โซเดียม ฟอสเฟต (Sodium phosphates)<br>โพแทสเซียม ฟอสเฟต<br>(Potassiumphosphates)<br>แคลเซียม ฟอสเฟต (Calciumphosphates)<br>ไดฟอสเฟต (Diphosphates)<br>ไตรฟอสเฟต (Triphosphates)<br>โพลีฟอสเฟต (Polyphosphates) | 2,000 ใช้อย่างเดียว<br>หรือ 3,000 ใช้ร่วมกัน<br>คำนวณในสภาพที่ปราศจากน้ำ  |

|    |                                |   |  |
|----|--------------------------------|---|--|
|    |                                | โซเดียม คาร์บอเนต (Sodium carbonates)<br>โพแทสเซียม คาร์บอเนต<br>(Potassium carbonates) |  |
| 4. | อิมัลซิไฟเออร์<br>(Emulsifier) | เลซิทิน (Lecithins)   | ปริมาณที่เหมาะสมตามความ<br>จำเป็นในการผลิต |
| 5. | สารทำให้ข้น<br>(Thickener)     | คาราจีแนน (Carrageenan)   | 150  |



## Appendix C

### Standard method for milk proximate analysis

#### 1. Fat analysis

The traditional standard methods for fat analysis are based on gravimetric method or volumetric method. They involve destruction of the globule structure of the butterfat, separation and then either drying and weighing of the fat or reading off the amount on a calibrated tube. The most commonly used methods are:

**1.1 Rose-Gottlieb method.** These methods, widely used for dairy products. They involve dissolution of non-fat solids in ammonia rather than the acid. The fat is then extracted using solvents i.e. diethylether and petroleum ether, with the addition of ethanol to avoid emulsion formation. The solvent is then removed and fat residue weighed.

**1.2 Mojonnier method.** Fat is extracted with a mixture of ethyl ether and petroleum ether in a Mojonnier flask, and the extracted fat is dried to a constant weight and expressed as percent fat by weight.

**1.3 Babcock method.** In the Babcock method,  $H_2SO_4$  is added to known amount of milk in the Babcock bottle. The sulfuric acid digests protein, generates heat, and releases the fat. Centrifugation and hot water addition isolate fat for quantification in the graduated portion of the test bottle. The fat is measured volumetrically, but the result is expressed as percent fat by weight.

**1.4 Gerber method.** The principle of the Gerber method is similar to that of the Babcock method, but it uses sulfuric acid and amyl alcohol. The sulfuric acid digests proteins and carbohydrates, release fat, and maintain the fat in a liquid state by generating heat.

#### 2. Protein analysis

The traditional standard method for protein analysis is the Kjeldahl method, which is also the most accurate. In the Kjeldahl procedure and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts.

The total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and distilled into a boric acid solution. The borate anions formed are titrated with standardized acid, which is converted to nitrogen in the sample. The result of the analysis represents the crude protein content of the food since nitrogen also comes from non protein components.

### **3. Lactose analysis**

The most commonly used standard method for determination of lactose is Polarimetric method. This procedure, which makes use of instruments known as polarimeters, is based on the principle that sugars such as lactose are optically active, i.e. they will rotate the direction of a beam of plane polarized light (light of one wavelength moving in one plane), the amount of rotation being dependent on the concentration of sugars in solution.

A second method of lactose analysis is an enzymatic method, which is specific for lactose. It is based on the enzymatic breakdown of lactose molecules into glucose and galactose. Finally, some carbohydrates including lactose can be measured by exploiting their ability to reduce other reagents, which are determined either gravimetrically.

### **4. Total solid (TS) and solid not fat (SNF) analysis**

The most commonly used standard method for determination of total solid is Air oven drying method. In this method, the sample is heated under specified conditions, and the loss of weight is used to calculate the total solid content of the sample. For solid not fat can calculate by difference between total solid.

## BIOGRAPHY

|                             |  |
|-----------------------------|--|
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